Webinar June 2020 – Bacteriatherapy for NF2 - Script .

Helaine Bader: We're going to get started. Welcome to the NF2 BioSolutions webinar on bacteriotherapy for NF2. We are so happy you can join us. I am Helaine Bader, a volunteer with NF2 BioSolutions, and I have just a few quick housekeeping notes before we get started.

So first, all participants will be muted during the webinar, and you'll be able to ask any questions through the chat function, but we will have the chat box locked until after the presentation, and then we will open it up for questions. If for any reason you are unable to get to your question or we don't have the answer, we will put together an answer document and send that out to all attendees in the next few days.

The webinar is being recorded, so that will be available on our website for future viewing. Finally, we would love your input on what webinar topics you would like to see in the future. So please feel free to enter your suggestions in the chat box as well.

Now I'd like to introduce our presenters. Dr. Gary Brenner is the director of the Massachusetts General Hospital pain medicine fellowship since 2002, and he currently sees patients at the MGH center for pain medicine. He is also an associate professor of anesthesia at Harvard medical school. Dr. Brenner's lab is focused on developing gene and cell-based therapies for schwannoma and other NF associated tumors. He has authored more than 50 articles, reviews, chapters, and abstracts on gene therapy strategies for NF tumors, the pathophysiology of pain, stress effects on immune function, and clinical approaches to chronic pain. Dr. Brenner currently holds several national leadership positions related to pain medicine education and training.

Dr. John Mekalanos is the Lehman professor microbiology and molecular genetics at Harvard medical school and served as chair of the department of microbiology and immunobiology for two decades, starting in 1996. His research spans multiple facets of

bacteriopathogenesis, with an emphasis on using genetic and functional genomic approaches to explore gene regulation and host pathogen interactions. His laboratory has provided many key genetic tools that have been successfully used in the field for decades and have defined the mechanism that's have led to bacterial virulence. Dr. Mekalanos has received many honors, including election to the national academy of sciences and American academy of microbiology, the Eli Lilly award, city of medicine award, the Santa Fe institute pasteur award, and the Drexel prize. He has been a member of the FDA advisory committee on vaccines and related biologics and has consulted for numerous governmental and private agencies.

We are thrilled to have both Dr. Brenner and Dr. Mekalanos here with us today. Dr. Brenner?

Dr Brenner: Thank you for the kind introduction. On behalf of John and myself, I want to thank you all at NF2 biosolutions to present what I genuinely feel is possibly the most exciting project I've ever worked on in my laboratory career.

I'm going to try to keep the talk quite conceptual instead of digging too deeply into the data. I will mention this is the first time I'm publicly presenting this data from our lab and the collaboration with Dr. Mekalanos.

So I always like to give a little bit of history to provide an underpinning for why we're doing what we're doing. Almost nothing that we do in science is something that isn't in part based on the observations and work of people who came before us. So there actually is a fair history of what's come to be known as bacterial cancer therapy.

In 1725, Antoine Deidier, a French physician, observed that tumors in patients with syphilis seemed to be cured more frequently, and syphilitic prostitutes appeared to have lower levels of cancer than the general population.

About 100 years later, an English physician, James Paget, suggested that an infection in one of his patients may have caused tumor regression. And then about the same time, a German physician, Wilhelm Busch found evidence of cancer regression following a post-operative infection. Now, these reports weren't widely disseminated. The watershed moment really occurred with American physician William Coley. He was an oncolytic surgeon, and in 1890 he treated 17--year--old Elizabeth Daschiell for sarcoma of the limb. The standard of care at the time was amputation of the affected limb. Unfortunately, Ms. Daschiell died ten months later from the same cancer which it metastasized. And as with many tumors today that involve resection, the results were unfortunately not what we hope with our patients.

After his experience, he reviewed New York hospital records and found a case of one patient, a Fred Stein, who had inoperable neck sarcoma that absolutely vanished following the bacterial skin infection with a Gram-positive streptococcus. He was so interested in this that he dug deeply into the literature, which wasn't easy at the time because there were no computers - reminds me of my Ph.D. training --- (and did) extensive research into the medical literature, and this is why I mentioned those other physicians; Deidier, Paget, and Busch. And he saw their reports. And that, in conjunction with Fred Stein and 47 other cases of infection associated with cancer regression, really got his interest, and he made this his life work.

He proposed, and really the first person to widely disseminate this concept that bacterial based cancer regression was, in fact, something that occurred not infrequently, and the result of actually anti--tumor immune responses. Imagine that, prior to knowledge of the cells of the immune system, he made this amazing hypothesis.

In 1891, he did something which naturally could not be done today. He injected an inoperable throat tumor with streptococcus, and the tumor resolved after multiple such injections. Back in those days, people often experimented on themselves, in fact.

In 1893, he did what was critical. He had an observation. He did some research. He proposed a hypothesis. He tested the hypothesis, and then he published. So he really did what any good scientist should be doing today as well. And he published "the treatment of malignant tumors by repeated inoculations of Erysipelas, with a report of ten original cases."

And he dedicated his life to developing a cancer therapy. Unfortunately, our understanding of bacteriology, of the immune system, of human genetics obviously was in its infancy, and he never really got to a truly effective treatment.

But much more recently, all of those factors have changed. In 1990, the FDA actually approved use of live bacteria, BCG, for non-invasive bladder cancer. And that treatment remains the standard of care today with a reported 50% cure rate. It's quite amazing. And the treatment is thought to represent an immunotherapy as these bacteria are highly immunostimulatory. The product, unfortunately, is produced only by Merck, and there's currently a worldwide shortage. It should be used with six applications, and currently it's used in three applications because of that shortage.

Since approval of this bacterial product, BCG, for bladder cancer, there's been increasing interest generally in bacterial cancer therapy. This has occurred because of development of genetically-modified bacteria that can improve both safety and efficacy of the treatment. There's a large, large number of animal studies that have used many different strains of bacteria and have shown value in treating multiple, different cancers. Of course, these are animal models of cancers.

There are, I'm aware of at least a few biotech startup companies that currently exist and are devoted to developing bacterial cancer therapy. And there are actually multiple clinical trials currently under way, though unfortunately none have yet led to another FDA--approved bacterial treatment for cancer, but I do think that's on the horizon.

Now, this is the key point and one of the insights that we're bringing to the table. Bacterial therapy, as far as I can tell (and I've worked deeply) has never been proposed, and obviously never tested, for the treatment of any benign or noncancerous tumor. Obviously, schwannomas are a benign neoplasm in virtually all cases.

So that's the history, that's the current update. Let's talk a little bit about why we think bacterial therapy for schwannoma and related NF tumors makes sense.

So how does bacterial cancer therapy work? There's direct killing of tumor cells. These bacteria can actually get into the tumor cells, and they have innate mechanisms that allow to kill those tumor cells. They also inhibit angiogenesis, which is they deprive tumors of their vascular blood supply, and without the blood supply, these tumors don't grow. As you know, the only drug that's really routinely used for a schwannoma now is Bevacizumab, or Avastin. And finally, these bacteria have known to initiate anti--tumor immune responses. They turn the host immune system on, and it allows it to attack the tumor cells.

So specifically for schwannoma, why do we believe that it makes even more sense, actually, than cancer therapy? Well, schwannomas are solid tumors with a hypoxic core, and these bacteria, the ones we're using, thrive in a low oxygen or hypoxic environment. The tumors are slow growing. So unlike cancers, these bacteria will have more time to replicate within the tumor and attack those tumor cells and more time to develop an immune response to attack the tumor cells before they can change, and they're highly vascularized. So for many reasons, we think this makes sense. And one

key point is that schwannomas are known to contain the immune system cells that are necessary for the bacteria to induce anti--tumor immunity.

And I'll just tell you one other piece, which is we think that schwannomas are what we call immunologically cold. There's a lot of suppression of immune responses, unlike cancers, which are hot, immunologically hot; a lot going on immunologically. And we believe --- and we have data to support this - that bacteria have the capacity to change the schwannoma from immunologically cold to immunologically hot and cause an immunologic effect that allow the host to start killing the tumors. So just to recap, bacteria tumor therapy, it's rational for schwannoma treatment.

We plan to deliver via direct tumor injection. It's a minimally invasive procedure. These tumors can be readily localized via MRI or ultrasound. If I didn't make this clear, these bacterial products have been used in humans, they've been used safely in humans, and they've even been used in humans via direct tumor injection.

As I mentioned, schwannomas are slowly replicating and genetically stable, and we believe this will enhance this therapeutic strategy, and they're, as I said, hypoxic, which means a low oxygen environment where the bacteria can thrive, and they're very vascular, which is another feature of the tumor that the bacteria can attack.

Our bacteria, again, has proven safe in multiple clinical trials. This is critical. So we only minimally need to worry about whether we're going to harm patients with the therapy. I think that's very, very unlikely, given the clinical experience.

And tumor killing occurs via multiple pathways, including, importantly, the induction of anti-tumor adaptive immune responses, so specific immune responses.

And I'll give you a little bit of a punch line because I'm not going to show you these data-- I'm going to try not to make you punch down the data today, as I said, make it more conceptual-- but we've shown in the laboratory that treatment of a single tumor

with direct injection of the bacteria controls growth of a tumor in another part of the animal. Secondarily, we have shown that treatment of a single tumor allows later on control of a developing tumor. So if we treat one tumor, wait, and then implant another schwannoma, the second schwannoma, which hasn't been directly treated, is actually growth-controlled better than the original tumor. It's quite amazing.

So I know many of you know these materials I'm about to present this slide back and forth. I don't mean to speak under anyone. I just want to make sure everyone on this call has the necessary background related to schwannoma.

Schwannomas, as I said, are non-malignant tumors that grow in peripheral nerves, but benign, I think, is a really bad word. I like non-malignant because they're anything but benign, particularly because they grow within the cranium, within the skull, and in the spine, and do horrible damage to patients.

They appear largely first in children and young adults with new lesions occurring throughout life. Further increasing the burden of disease. Multiple lesions are generally or essentially always present unless it's sporadic schwannoma, and as I've alluded to, the tumors cause debilitating neurologic disorders, including severe persistent pain. I became interested in this field because of my treatment of patients with pain. I work solely in a pain clinic clinically, and there's substantial psychological co-morbidity, as you know, not just for patients, but for their family members.

And the current standard of care, resection -- usually operative and to a lesser extent with radiation, radiotherapy -- is itself associated with significant damage to the nervous system, and some of these tumors, because of their location, can't be removed surgically because it's just too dangerous.

So in order to test our hypothesis or our idea that bacterial therapy for schwannoma makes sense, we needed animal models, and unfortunately, there aren't a lot of animal

models for schwannoma. We actually developed two in order to test the therapies, the gene therapies and this bacterial therapy.

What we do is we take human NF2 schwannoma cells and directly implant them into a peripheral nerve of the mouse, and you can see that here, and this is just a glass catheter we use to inject the cells. And the cells grow just fine on their own. We've also genetically modified the tumor cells so that, when we add Luciferase, the cells light up, and with a very sensitive camera, we can basically see easily how many cells there are. Here are two animals with very few number of tumors, and here are two animals with a large tumor burden. So we can follow animals across time while their tumor is developing and do a treatment, in this case, inject bacteria directly into the tumor, and see what the effects are.

So these were the first data we obtained, and it was kind of one of these “oh, my gosh” moments, like is this real? The red line is the control injected tumors. These are eight animals in each group. And the green line is the bacterial injected tumors. What you can see is there's an unequivocal effect that's lasting as well. We are developing a gene therapy, which I'm already very much excited about, and we're trying to get it to clinical trials. I'll tell you, we never saw anything of this magnitude.

And you always have to repeat your findings, and this was our second experiment, and we exactly repeated the findings of the first experiment. We do these experiments in a way that's called blinded, so we don't know which animals are which. That's to remove any bias. In three independent experiments, we found that 75% of the bacterial-injected tumors fully resolved. They just melt away. This is a very unusual finding in the bacterial tumor therapy literature.

We wanted to look at a second model in the cancer therapeutics field. One model is always considered inadequate, and I agree with that. So this is a completely different

model. It's a different mouse, and in this case, we implant a mouse, not a human, NF2 tumor line, and we find that these cells grow a lot faster. You may not have noticed that before we were looking at weeks on this axis. Here are days. But we see essentially the same effect. The control animals, their tumors grow and grow and grow. In fact, we have to sacrifice them because the tumors get so big and it affects their nerve function. But the bacterial treated animals, the tumor burden is kept much smaller. Very exciting data to us.

But we need to know how it works. We need to have a mechanism. I'm going to run through these data fairly quickly. If someone had real interest in seeing the data in depth, I'm happy to share it. We are about to submit our first paper for publication. First, we found that bacterial injected tumors undergo what we call apoptosis or program cell death. They actually undergo several types of cell death, but certainly apoptosis, and here you see bacterial and control treated tumors. These are slides showing this, and a stain for apoptosis and you see a lot in the bacterial treated tumors, really a tremendous amount, and very, very little in the control samples in both the models.

We also find in the bacterial treated animals, there's inhibition of blood vessels, and here are the control treated tumors - and trust me, when you look at them under the microscope, they're big, red, and full of vessels, and we see a lot of vessels with staining. And here are the bacterial treated tumors shrunken, white, and very little evidence of vessels. It's very exciting. Honestly, it's what we would have expected from the bacterial cancer literature.

There's also infiltration into the tumors of immune system cells, both lymphocytes and macrophages, I won't belabor the staining, but there's a great increase -- and I'll get into that in a minute -- in the resident immune cells, and we think that's very important in

generating the immune response. Perhaps even more interesting than the increased number of immune cells is what I'm about to show you.

Macrophages, which are key cells in generating immune responses, come in two flavors inside of tumors. One is tumor promoting, they're called M2, and the other are called M1 or tumoricidal. The names are obvious. Resting schwanns, untreated schwannomas, seem to have M2 type macrophages. After the treating, the green bars, day 3 and day 7, what you see is a shift in the control red to -- from M2 to M1 tumor killing macrophages. This is a very exciting finding because these are key cell types. Some of you, I'm sure, are familiar with different kinds of lymphocytes, which are what we call the effector cells in these processes, and as I mentioned, schwannomas have lymphocytes. Here's the control, red bars. Unfortunately, in the untreated tumors, as in humans, most of the lymphocytes, there are not many killer lymphocyte that's would kill tumor cells, but there are a lot of what are called T-REGS, or regulatory T cells that actually stop the body from being able to generate anti-tumor responses.

Now, after bacterial injection, green bars, an amazing thing comes, happens. You vastly increase the number of tumor killing T-cells, and you decrease the regulatory T-cells. So exactly what you want to see if you were designing a therapy, and I'll tell you, we also see increase in something called helper T--cells, and they're another lymphocyte type that can facilitate immune responses.

It's always important to look for toxicology or damage from any treatment, and I'll just tell you that with very sensitive sensory testing in these animals, we see no effect of bacteria on the animal's behavior, and we also see no effect on motor function. So these data in animals mimic what we expect to see from human data, which is these bacteria are safe. They don't cause problems on their own. Naturally, we have to think a lot about the tumors in the spinal cord or in the cranium, in the skull, but we do think

there's ways we can design these bacteria to be so safe that it will even make sense to inject them directly into tumors in the cranial wall.

So a summary, bacterial tumor injection of schwannomas, a novel schwannoma immunotherapy. Safety has been established in early phase clinical trials, two intravenous and one injection with exactly the product we're using in our animal studies. We've shown efficacy, i.e. it works, in two completely different schwannoma models and replicated the findings. We've shown that there's tumor killing via apoptosis, programmed cell death, vessel growth control, and most important, what we call immunogenetic cell death, which is a form of cell death that causes anti-tumor immune responses. And these are the shift to tumoricidal macrophages, increased cytotoxic and decreased regulatory T--cells. And these are what we believe are leading to the induction of host anti-tumor adaptive immune responses, what we would generally call a vaccination effect.

This has the potential to control growth of multiple tumors following bacterial treatment of a single schwannoma and the potential, which hope, for lasting anti-tumor immunity following treatment that could control the growth of subsequently arising tumors.

I think now you know why we're so excited about this. And I haven't shown you this today, but we've enhanced the effect of the bacteria on anti-tumor immunity with the use of immune checkpoint inhibitors.

So I don't want you to think this would only work for schwannomas because NF2 patients have other types of tumors, and we also care about other benign neoplasms, obviously. I would just briefly show you we have efficacy data with the same product in benign meningiomas, and the effect is really quite obvious and lasting. This is 41 days, which is quite a long time in animal studies. And we even looked at an NF1 associated

tumor. This happens to be a malignancy, a peripheral neuro sheath tumor, but, again, we see a quite clear effect on tumor growth. This adds to our excitement in the project. And while we're using an existing bacterial product that may, in fact, work very well in humans, we, at the same time, are working on ways to enhance bacterial induced schwannoma and other tumor cell killing because we know these products haven't worked in human beings for cancers. Again, schwannomas are fundamentally different, as in meningioma, but we want to more or less hedge our bets to get to a real treatment.

So what are we doing? We're reengineering this bacteria to enhance anti-tumor immune responses. So we can engineer super adjuvants into bacterial cells of a variety of types, and what I'm showing you here is something that's relatively recent out of the lab, where the bright light dots are actually our bacteria that we've reengineered to express a protein called GFP that lights up. What's amazing is you kill all the bacteria outside the tumor cell, so everything you see are bacteria inside tumor cells. Maybe you can kind of see this is a tumor cell, and it's chockful of bacteria in there. And I will also say we've engineered with protein, GFP, to be secreted by the bacteria. It's very exciting.

So we're at the same time, actually right now, waiting to hear from someone. We're working to engineer the bacteria to express molecules that would specifically enhance anti-tumor immunity. It's all very exciting.

And working with John's lab, we want to eliminate bacterial properties that might inhibit development of anti-tumor immunity. At this point, I want to turn the talk over to John, but before I do that, I just want to express to the audience how excited we are to have John as part of our collaboration. You heard the intro, but I don't think it really gives you the true flavor of the fact that John is a giant in the field of bacteriology, and I couldn't

imagine a better person to work with to help us reengineer these bacteria. With that, I'll turn things over to you, John.

Dr. Mekalanos: Okay. Thank you very much, Gary. I really appreciate it. I want to emphasize again, despite the accolades and compliments, I've never worked on anti-tumor bacteria, if you will. This is a new area for me. I feel like my whole life - 40 years plus of research - has been devoted to understanding how bacteria interact with cells in the immune system, and we've made some pretty important contributions along the way, but when I was approached by Gary and his colleagues to consider getting involved in this collaboration, I was seriously very excited to see these data and realize, wow, this is just perfect timing because we have a lot of new technologies that could potentially improve the potency and the efficacy of this bacterial-based therapy for schwannoma, and schwannoma was, as I learned, was such an unmet medical need. The field largely dominated by all kinds of other inoperable neoplasa of so many varieties that aren't responding to checkpoint blockade or aren't responding to other therapies. So having a chance to hone in on Gary's guidance on fantastic animal models and fantastic preliminary results, it was just an opportunity that I couldn't pass up.

But as I said, I don't work on the tumor models in general in my laboratory, so my focus of making a few comments is just going to try to give you some excitement about where we are in the bacterial pathogenesis field because we feel we're learning so much about how bacteria interact with cells in both positive and negative ways, if you will. Positive being kill a cell, but negative can be anything from causing host damage to suppressing an immune response. So all these things are important when you think about a

bacterial--based therapy. You don't want the bacterium to interfere with the immune response, but you may want the bacterium to be more potent at killing the tumor cell. And if I don't remember to say it, I'll say it now once, and I've said it at least once. There is an emerging strong literature that says that, when bacteria kill tumor cells or a mammalian cell, that alone can be very immunostimulatory. As Gary pointed out, the schwannoma cells can undergo apoptosis. There are other types of cell death, myriad names; necrotic cell death, pyroptosis, et cetera. And we're starting to understand the molecules that drive cell both in different directions, and those cell death pathways actually have tremendous consequences for the immune response of the antigens that are released, which would include tumor specific antigens. With that said, I won't forget to say it at the right time.

Gary, if you'd change the slide. So where we're going to focus is we're going to focus on enhancing direct tumor killing by bacterial cells, and we have a variety of ways to go about doing this. One of the primary ways we're very interested in doing is we devised methods in the lab to allow bacteria to target specific cells. These are genetic methods where we can kind the needle in the haystack, and we're really excited to apply this methodology to bacteria, first, the bacterium that Gary has the most experience with, but eventually a completely reengineered strain, because I think there are advantages in that as well, and target those bacteria to schwannoma cells with very specific genetic methods.

Our prediction is that's going to improve the specificity, perhaps even the potency of the bacteria in the therapeutic models that Gary devised. We'd like to improve invasion of tumor cells. Certainly, these bacteria invade the tumor cells, as Gary pointed out with the TFP image in the upper right hand corner, but it's possible we might be able to get

more, quantitatively more invasion by decorating our cells with molecules that specifically bind schwannoma cells.

We also feel that invasion is an interesting property, bacteria have multiple, different ways they can enter inside a cell, and some of those pathways are immune suppressive, and some of these pathways are immune stimulatory. So by engineering the right immune cell pathway might, again, greatly bring the potency of bacterium for killing the tumor cell and also activating the immune system.

We also are very interested in all kinds of virulence factors, but I mention this one because you'll see beautiful videos in a second. Bacteria produce very powerful cell killing machines, and we call these secretion systems because they literally take proteins and inject them into a target cell -- in this case, a tumor cell -- using a Nano machine. Think of it as a very small syringe, just Nanometers in size, really one-tenth or one--hundredths the size of a bacterial cell.

So with these very powerful little syringes, if you will, bacteria can inject very potent killing toxins, if you will, molecules that have been designed or otherwise are known to kill cells in a very specific fashion. We know a lot about how to do that and have designed these systems from the ground up.

So on the next couple of slides, I'm going to show you examples of how phenomenal these killing machines are. What you're looking at now is the cells, just think of them as good cells. The red flashes you see are one of these Nano machines, secretion machines called type 6. The dark cells, if you will, are bad cells. They happen to be E.Coli, but in this model, you can see the red cells are firing away their little machines, and watch what happens to the E.Coli, good-bye. So bacteria can use these machines to tell other bacteria that they're competing with, and that's interesting ecologically, but it has applications, and we've already developed one application where we're using a

good bacterium to protect animals that are commercially important in the food industry from bacteria that are bad, using these kinds of killing machines. So this idea has been kicking around, but now we're really starting to use it for applications.

We'd love to be able to do this specifically with tumor cells, specifically schwannoma. Next slide please, Gary. The next video you're going to see gets a little busy after a while because the bacteria are growing while everything is going on.

So I just wanted to briefly focus in on a couple of things. First of all, the big cells that you see on the field arrowed out in red (yes, exactly, Gary)-- these are tumor cells, and the tiny cells that you see, with a white arrow up at the top, those are bacterial cells. You can see tumor cells are literally a thousand times the volume of a bacterial cell, but we've been able to show that even a single bacterial cell can kill that tumor cell using these kinds of machines.

What you'll see is also a phenomenal thing that goes on now is that the bacteria that we're going to use to attack these cells have the machine marked with a protein called green fluorescent protein. So the bacteria will turn green under a fluorescent microscope when they turn on the killing machine, and what is really amazing about this is the bacteria are so smart already that they don't turn the machine on right away. They have to bind to the target cell. Binding is enough to activate a pathway that the bacterium senses and says now it's time to turn on my killing machine, and that's going to be indicated by green cells, and you'll see that the green cells that are very close, if not stuck, to the tumor cells are the ones that do the job.

So if you run the video right now, you'll see it in action. This is time lapse, so the pictures taken a little hurky jerky, every 15 minutes, as I recall, a snapshot is made, but it's turned into a video. You saw where the bacteria were. They're merely growing and binding to cells. They just keep on going. All of a sudden, some of the ones that have

bound start turning green. You can see if you look across the whole field, there's lots of bacteria, but where are the green ones? The green ones are right next to the tumor cells that are no longer tumor cells, okay? That's the amazing thing is what's left here are just bacteria stuck to what's called cytoskeletal components. These are the leftover Dregs of what is more or less a vaporized tumor cell.

We can imagine that, when that tumor cell lights, all kinds of stimulatory models were released from the target cell, but more importantly, this demonstrates that it may even be possible to very specifically kill tumors without the bacteria ever even entering the tumor cell, and that might have consequence for the type of immune response you get. So both invasion, being able to make the bacteria enter tumor cells more efficiently, as well as preventing invasion but still killing tumor cells, it needs to be investigated. And I think we have the preclinical models through Gary's collaboration, to really tease out, at least in the mouse model, which of these properties correlate best with immune responses, and in that adds to our information on how to devise a better human therapeutic agent, we will be delighted. We seldom have a chance in our basic research to have such a great shot on goal here for applied science, and I think we can get this into the clinic pretty quickly using the models that Gary has described.

I'll stop there and pass the ball back to Gary to finish up.

Dr. Brenner: Thanks, John. It's a pleasure to work with someone as humble as you. What you failed to mention was you discovered one of these key bacterial mammalian cell killing machines, and it's really an honor to work with you, and I mean that.

Dr: Mekalanos: Well, thanks.

Dr. Brenner: So I want to give some credit to Sherif Ahmed, who's the key scientist in my laboratory, and it was really Sherif and myself who came upon this concept of

bacterial therapy for schwannoma and other benign neoplasms. I'll just mention that it was a direct offshoot of the biology of the gene therapy we're doing, so they are related. But I don't believe any great discoveries or work comes out collaboration. I welcome collaboration. Personally, working for what John alluded to, which is to translate work from the laboratory to a treatment which helps people.

My life goal - and I'm sure John would say the same --- isn't to better treat disease in mice and rats. It's to find something that helps patients. It's a great need. I will also mention that we have other members of our collaborative work. I work with immunobiologist, her name is Shin Fei Wong. And I work with a number of surgeons because, as you'll hear in a moment, we want to work with human tissue as soon as possible to help us better design the next therapeutic, the improved bacteria that John discussed so eloquently.

So what are the next big steps? As you heard, this is really currently getting started, but it will be the major push in the near future, which is genetic engineering and animal testing to develop more effective -- and I would add safer -- bacteria with fail safe mechanisms but better strains at the end of the day. We want to investigate the biology of these improved bacterial products in fresh resected human schwannomas. The fact is there's only so much you can learn by looking at these animal tissues. There are fundamental differences, both between the tumors and the host immune responses in animals and man, and in order to be smart and make the right product that has the highest chance of being effective and the highest chance of being safe, we need to look at human tissue as soon as possible.

And we hope to use those two -- the information from those two above to obtain the large amounts of money through federal funding to move to early phase clinical testing with the current product, use the information. I mean, if it's a home run and it happens

to work and it's safe, no one would be happier than I. But I think we have to go into this with the understanding there's a good chance the current product might not be optimal, but we can use that information from those studies to help us design the product that will be the therapeutic, we hope.

I think that's really all I have to say. John, do you have any summary comments?

Dr. Mekalanos: No, I think you echoed my feelings exactly. I think we all know phase 1 human testing, development of a product for real -- to have it on a shelf and available for treatment, it's going to take more data and more funding, but we are well on the way, I think, in this collaboration to being able to generate both of those with a little bit of luck.

Dr. Brenner: At this point, I'll turn things back to Helaine, I believe.

Helaine Bader: Yeah, so thank you both for such an amazing presentation. That was wonderful. This is really exciting research. We do have the chat box open for questions. So please feel free to enter your questions there, but we do already have a few questions for you. So we'll just get started and dive right in.

The first question, as you mentioned, 75% of bacterial injected tumors fully resolved. Did the treatment shrink existing tumors or stop and slow the growth of new tumors after inoculation?

Dr. Brenner: That's a great question. In a human tumor model, they actually shrunk and made the tumors disappear. There was no signal. When we looked at the nerves, they were quite normal. In the mass schwannoma model, the tumors, some shrunk and some slowed. The overall effect, when you look at averages, was decreased tumor size, but we didn't see those tumors completely go away.

Interestingly, in what we call in that same model, the rechallenged tumors -- so that's where we have one tumor growing, we treat it, we wait, in this case, about two weeks

and implant more tumor cells, those, half of those tumors completely resolved. So that's a great question.

Helaine Bader: We also got a comment, this has been a fascinating talk, thank you. And a question, can you please discuss more the situation involving the stop break on the immune response? If the immune response is not stopped, then autoimmune and inflammation results. Where is the science as far as this is concerned? And how difficult will it be to put this sort of treatment into someone without the proof that the response can be shut down?

Dr Brenner: Sure. So the fact of the matter is we don't know at this time which antigens in the animal models are the basis for the anti-tumor immune response. Having said that, we don't see any evidence of either motor dysfunction or sensory dysfunction, and with the sensory tests we are using, we can see what we call gain of function and loss of function effects. So pain -- for sensation, we could see pain, we could see decreased sensation. So at least in the animal models, we don't see the development of autoimmunity.

Having said that, it is an important consideration. In the bacterial cancer therapy trials that have occurred thus far, I'm not aware of any cases of autoimmunity occurring. It is possible, and what we would hope is that the immunity is based on the tumor specific antigen. In this case, it would be unlikely to develop autoimmunity. But, again, I want to say to the person who brought this up, it is a very important consideration, and in developing clinical products and testing them, one of the most important consideration, if not the most, is not making the life of the person you're testing something on any worse than it already is.

Dr: Mekalanos: I think Gary did a great job of answering that question from the point of view of autoimmunity perhaps driven by now a new immune response against the

schwannoma antigen. Obviously, we wouldn't want B and T-cells running rampant and attacking healthy schwann cells throughout the body. But the other thing I picked up on is the checkpoint effects. Gary has very convincing data that one type of immune checkpoint blocker, if you will, an antibody that blocks a very critical molecule in the checkpoint response --- I think it's PDL or PDL1. But anyway, it's being used in many cancer treatments successfully. It seems to enhance the anti-tumor response.

So I would say, from that point of view, it sounds to me like it's classic checkpoint blockade controlling a very potent immune response against the schwannoma, but even with that, Gary, those experiments showed no toxicity, no autoimmune, no systemic autoimmunity, as best you could tell.

So I think that for transient treatment to block the checkpoint, as one example of sort of an adjuvant therapy of trying to enhance the immune responses, most molecules we envision are not going to be chronically on board. They're going to be transiently used in the context of immunotherapy, and eventually they will go away, and we certainly don't expect any worse toxicity than folks are seeing with these molecules in standard cancer - not that that is - you know, some of them have toxicity problems, like CTLA-4, you can look up, has a lot more toxicity associated with it but does a lot of the same stuff.

So I think we need to investigate that when the time comes. Gary, perhaps you can go one step further and say that would be kind of the next phase. We have a successful therapy working pretty well in phase 1 or phase 1A, B, extended studies. Yeah, you can take those checkpoint molecules right off the shelf and combine them. So I think the follow-up clinical trial could be happening very, very quickly.

Dr. Brenner: Thanks, John. That's certainly part of our plan if it's necessary to use checkpoint inhibition. We would roll that into a clinical trial. If the human schwannomas

do have PD1, PDL1, and CTLA for based signaling, we have some data to show that, which is why we modeled that in the mouse. Unfortunately, the mouse model also has the PD1 pathway.

And one advantage there is, if the checkpoint inhibition is necessary for efficacy, if there were autoimmunity, it is possible that removing those molecules from the system would deal with the autoimmunity as well. So we are definitely thinking or doing our best to think a few steps ahead, and as John alluded to, combination of cancer therapeutics with a standard agent and a checkpoint inhibition is really very common, and I suspect might be the direction we eventually go.

Helaine Bader: Great. We have a bunch of questions. So we'll try to get to as many as possible. We have a comment, really cool project, and then this is a multipart question. So how many times were the mice treated? And were the bacteria directly injected into the tumor? Did you also try systemic treatment? And did you look at the life span of mice with the mouse tumors that were treated with the bacteria?

Dr. Brenner: All good questions. So the tumors are injected once directly. We actually have two different tumor models, even with the mouse model. One is the cells are in the nerve, and I didn't mention this, but the other is a subcutaneous tumor, to allow the animals to survive longer.

We haven't done a...we haven't conducted a survival study yet. It's something that we may do at some point. I don't know if it's truly valuable in the context of the model systems we're using. The effect size in terms of tumor regression or tumor control is so great that -- I'm just not sure what value in animal models would be derived from survival studies, though, as the questioner rightly points out, that is a very standard question that's asked in preclinical cancer models.

Helaine Bader: And have you contemplated using another animal model that may be closer to a human like a swine?

Dr Brenner: Yeah, so the swine - of course, yes. The swine, NF2 swine, as far as I'm aware, are not yet fully characterized and not yet available for preclinical testing. I would love to try it in one of these animals when they become available. I would mention that pigs cost $10,000 apiece. There's an NF1 pig, as I'm sure you're aware, and then animal housing becomes more expensive.

I do think it has value, but on the other hand, we have a bacterial product that's been repeatedly shown safe in human trials. I really think it probably makes more sense to skip large animal models and go right to humans, if that's possible.

I think there was one other component of the previous question that I missed. Is that correct?

Helaine Bader: The life span of mice with the mouse tumors?

Dr. Brenner: Was there something before that?

Helaine Bader: And did you try systemic treatment?

Dr Brenner: Yes, that's right. So we haven't utilized systemic injection. That has been used in humans and mice, as we've alluded to. There's a reason for that. It may be that part of the reason that these treatments failed in human trials is that the tumors weren't colonized with the bacteria. Human beings seem to be quite a bit better at clearing these bacteria, the ones that we're using. Obviously, I'm being a little discreet about that. That won't last long. Because the human immune system clears them better.

So my thinking was it would make more sense to use a direct tumor injection that allows a higher inoculum or higher number of bacteria to be injected and thus increase the

likelihood of colonization, and there are some published human data that would very clearly indicate that that's the case.

I will depend on John to think about and help us engineer bacteria that are more capable of colonizing tumors without increasing the likelihood of causing systemic infection and significant toxicity to the patient.

Helaine Bader: Can you share what the bacteria used in this research was and why you chose it?

Dr Brenner: I can share why I chose it, which is a large preclinical body of data that indicated these bacteria can kill by all the mechanisms we discussed. A variety of forms of cell death, including immunogenetic cell death that leads to anti-tumor hosts, immune responses, control the blood supply, and changes in the innate immune cells in the tumors.

The reason I'm not sharing the exact product is because we do have a patent submitted. It should be published within a couple months. Once that occurs, I'll be free to openly share that exact product. As I mentioned, we're very close to submitting the first publication on this, which would obviously include the product. The other reason I chose to use this bacterium, which I think we've discussed several times, is it's been what we call de risk in clinical trials. That is, both by intravenous injection and direct tumoral injection, it can be used safely.

Helaine Bader: Great. So in the interest of time, we do have a number of questions that will go unanswered right now, but we will definitely reach out and be able to answer these later, but one question that keeps coming up -- and I'm sure you know what it's going to be -- but it's basically the question in different ways of asking what will happen next? Are there clinical trials happening? What's the biggest roadblock to getting it to clinic? How long will this take? What do we need to do to get this moving along?

Everybody is very excited for this. So I wanted you to be able to address that, and then we can have our next presenter come on to talk a little bit more about what's next.

Dr. Brenner: So the fundamental roadblock to clinical trials is funding. NF2 BioSolutions, Nicole and several of her colleagues, some of whom are on the webinar today, have done an amazing job of hooking us, the scientific researchers, into a variety of resources -- surgeons across the country, FDA experts, and other important resources -- that are helping us on this journey to develop a better product and get to clinical trials.

I think we have all the pieces we need right now, and I'm sorry to say this, but the piece that's lacking is the funding.

Helaine Bader: So with that, we are going to actually turn it over to Mike Halloran. Mike is a volunteer for NF2 biosolutions and the founder of two twin daughters with NF2. He has been an executive in the for profit and nonprofit space. So we'd love to hear from you, Mike.

Mike Halloran: Great, thank you so much, and I'll just take a minute because I know we're right up against the one hour time frame. I don't know how you all feel, but is that exciting or what? Just as a parent with daughters with NF2, to hear the results from Dr. Mekalanos and Dr. Brenner are really exciting seeing those green bacteria eating the red. The bad ones. I told Dr. Mekalanos, that's like a mic drop. The possibilities from this bacterial research is just tremendous.

So my wife and I are delighted to be volunteers to help out Nicole and the NF2 biosolutions just as volunteers. The question is about the next steps. The next steps are to fund this next trial. It's $100,000 to take this to the next trial and be able to fund the next trials that will then allow them to take it so the preclinicals, allow it to take it to the final clinical and the human trials and hopefully to a cure.

So we've set $100,000 to raise through the course of the summer. We're calling it the axe out NF2 campaign. It will go directly to this bacterial tumor therapy research to take it to the next phase, and we're really excited about it, and we think we can do it. We need support and help, advocacy. So really asking all of you - I know there's 85 on this webinar - to do two things, three things really. One, to be an ambassador and advocate for this research for NF2 BioSolutions, what we're doing, because what we're doing for NF2 tumors, as you heard from our two physicians, could really have applications to other diseases, and I'm learning all of this, and it's really exciting.

Second is to consider a financial investment in this Axe Out NF2 campaign, and we want to raise right off the bat $50,000 and use that as a challenge match, to then go to a broader public and do a dollar-for-dollar match here in July and August. So really focused on first reaching that first $50,000.

And then the next piece is to, as an ambassador, refer us. Let us know if there's others that should hear this story and might be interested in supporting this campaign and this research. On your screen, there you see our little visual for our campaign as well as the NF2 biosolutions. There's a lot more information on all that NF2 biosolutions, and Dr. Nicole Harwood founded it. She's a physician. She's amazing and her board, the work that's being done there. And our campaign for axe out NF2, there's a web link there. Also, I'll just give my e-mail, and maybe this could be typed in and send out as a recording, but Michael@NF2biosolutions.org. If you've got a direct question and you want to ask about support or advocacy, you can e-mail me directly.

Thanks for letting me take a little bit of time at the end here.

Helaine Bader: Thank you. Thanks so much to all of our presenters and for everyone attending. We will send the recording out within the week. We will also send a transcription out. We are sorry we couldn't get to everybody's questions, but we wanted

to respect everybody's time. We will go through and make sure all of those questions are answered, and we will send out a document so that no question will go unanswered. Please remember to visit WWW.NF2biosolutions.org. E-mail us if you have any questions. Please donate to this incredible research, if you can. We thank you for your interest and your contributions, and have a wonderful evening.

Dr Brenner: Thank you very much.