CURE Epilepsy Seminar

Tango – A Novel Approach to treat SCN1A-linked Dravet Syndrome

Lecturer: Dr. Lori Ipsom

(Transcript)

Priya Balasubramanian: 00:00 Welcome everyone. I'm Priya Balasubramanian. I am associate director of research at Cure Epilepsy. I'd like to thank you all for joining us today. As we celebrate Epilepsy Awareness Month this month, today's virtual seminars entitled, Targeted Augmentation of Nuclear Gene Output or TANGO, a novel therapeutic approach to treat SCN1A-linked Dravet Syndrome. The seminar will provide an overview of TANGO and present results from testing in mouse model of Dravet Syndrome.

Priya Balasubramanian: 00:33 This is the third and final virtual seminar of 2020 as part of our Frontiers in Research Seminar Series. This program is generously supported by the Nussenbaum-Vogelstein family. It aims to help educate and expose researchers, clinicians, and students to exciting epilepsy research, and also provide opportunities for young investigators to interact with leaders in the field. Because of the social distancing guidelines CURE Epilepsy has been unable to provide this interaction live at academic institutions around the world, so until conditions allow us to come back together, we will continue to present these seminars virtually also in 2021.

Priya Balasubramanian: 01:16 CURE has been proud to be a leader in the epilepsy research community for over 20 years funding over 240 projects spanning 15 countries. Right now we have three different funding mechanisms. Our key priority research areas include the acquired and pediatric epilepsy, treatment resistant epilepsies, sleep and epilepsy, and sudden unexpected death in epilepsy or SUDEP. All of our grant applications progress through a letter of intent phase and a full proposal review by scientific reviewers, as well as members of our community who were touched by epilepsy.

Priya Balasubramanian: 01:55 The catalyst award is our newest award. It is intended to fund translational research that aims to advance therapies into clinical application. Our CURE Epilepsy award is open to established investigators while the Taking Flight award is intended to support junior researchers who have at least three years of postdoc experience, but have yet to attain significant funding. The call for letters of intent for both the CURE Epilepsy and the Taking Flight awards will be coming out soon in the upcoming weeks. You can look out for that.

Priya Balasubramanian:	02:30	Today's presenter is Dr. Lori Isom. She is chair of the Department of Pharmacology, the Maurice H. Seever's professor of pharmacology, professor of molecular and integrative physiology and professor of neurology at the University of Michigan Medical School. Dr. Isom's research interests are to study the pathophysiological functions of human gene variance in developmental and epileptic encephalopathies, especially variants in genes that code for voltage gated sodium channels and to understand their role in epilepsy, cardiac arrhythmias and SUDEP. In addition to using transgenic mice and rabbits models, Dr. Isom's team is interested in using neurons and cardiac myocytes derived from patient specific IPSCs to study these variance.
Priya Balasubramanian:	03:17	Dr. Isom is a recipient of the 2015 CURE Epilepsy SUDEP research award. Her research using patient specific IPSC derived cardiac myocytes identified potential mechanisms by which known epilepsy genes can affect heart function and SUDEP and suggest that these myocytes may be a useful model to study SUDEP mechanism.
Priya Balasubramanian:	03:40	Before she begins. I would like to encourage everyone to ask questions. You can submit your questions anytime during the presentation, by typing them into the Q&A tab located at the bottom of your Zoom panel and click send. We'll do our best to get through as many of these as we can. I also want to mention that today's virtual seminar and all of our future seminars are recorded. They are available on the CURE Epilepsy website. With that, I will turn it over to Dr. Isom. Thank you.
Dr. Lori Isom:	04:09	Thank you, Priya. It's just my honor to be here. I'm honored to be selected for this really nice seminar series. I'd like to thank CURE for this invitation. I'm really excited today to share our work, which may provide some novel therapeutic approaches for patients with Dravet Syndrome and their families. You might notice that during the seminar for non-scientists, I know there's some non scientists in the room, that I've added some words, summary slides at certain places to catch everyone up to speed, but please feel free to ask questions if you don't understand. Let's start. Oops, wait a minute.
Dr. Lori Isom:	05:00	There we go. Before I begin, I'd like to disclose that this particular work was funded by a research grant to my lab from Stoke Therapeutics and that this work was done in collaboration with Stoke Therapeutics. My lab at the University of Michigan is made up of a wonderful group of very talented investigators from research faculty to postdocs to graduate students to undergraduates who are all working together to solve problems

and to understand the mechanisms of epileptic encephalopathy and to develop biomarkers and strategies to prevent SUDEP.

Dr. Lori Isom: 05:46 We all know on this call that Dravet's Syndrome is a devastating disease. I'm privileged to serve as co-chair of the advisory board for the Dravet Syndrome Foundation. Through that, I'm able to interact frequently with families of patients with Dravet Syndrome. This montage was made by a parent of a Dravet Syndrome patient to try and express the daily life of what they go through in their family.

Dr. Lori Isom: 06:20 Dravet syndrome is more than seizures. It's a rare and catastrophic developmental and epileptic encephalopathy that affects virtually every system in the body. It's a rare disease. It's actually not as rare as we used to think, but because of better diagnosis now, Dravet is found in about one in 16,000 patients. It includes an addition to severe seizures that start in usually the first year of life, behavioral and developmental delays, autism spectrum, difficulty swallowing, with speech, with sensory perception, with sleep, with ataxia and maybe the most devastating aspect of Dravet Syndrome is that it has up to a 20% risk of SUDEP or sudden unexpected death in epilepsy, and in spite of lots of innovative new, small molecules that have been introduced into the clinical realm, it remains pretty intractable.

Dr. Lori Isom: 07:31 Dravet Syndrome is largely caused by variants in the gene SCN1A that encodes the voltage gated sodium channel alpha subunit Nav1.1. This is a cartoon showing the typology in the plasma membrane of Nav1.1. This slide was made about 10 years ago by my colleague at the University of Michigan, Miriam Meisler, but it serves the function really well, because today there are over a thousand variants in SCN1A that are linked to Dravet, but if we put them all in here, we couldn't see the protein. This one really illustrates the point here.

Dr. Lori Isom: 08:12 The question early on for us was how is it that a variant that affects the end terminus of Nav1.1 can cause Dravet and so can a variant that's in that C terminus or anywhere else in between. For awhile, for sodium channel biologists this was a problem until we understood that the for the majority of these variants, the mRNA is targeted for nonsense-mediated decay. The patient has one wild type variant and the other variant just goes away into the trash. We learned that 50% of wild type SCN1A is insufficient for normal neuronal function.

Dr. Lori Isom: 08:59 Now I want to briefly go over what we know about the mechanism of Dravet syndrome. It's complex. There's a lot to understand. The very first model, a mouse model, of Dravet

syndrome was made by my former mentor, Bill Catterall at the University of Washington. He made a knockout or a null deletion, a deletion of a variant of SCN1A. He discovered that the heterozygotes that had one wild type allele and then the other allele was deleted had seizures and SUDEP. Then when they isolated neurons from hippocampus at postnatal day, or P, 14 and measured sodium current, they saw something that was unexpected to them. This is a family of sodium currents. We're looking at current density on the Y axis and with membrane potential on the X axis. They really saw no difference in this curve between all three genotypes, the wild type, the HATs or the nulls.

Dr. Lori Isom: 10:14 But what they did see differently in the bipolar or the GABAergic interneurons was a selective loss of sodium current in the here, you can see it in the heterozygotes and in the homozygotes compared to the wild type. The heterozygotes are the disease model. At that time, they proposed at this decreased sodium current in bipolar neurons, but not pyramidal neurons resulted in disinhibition. That's how epileptogenesis started.

Dr. Lori Isom: 10:46 Then a few years later, our colleagues Jennifer Carney and Al George made a similar model, mouse model, and they looked about a week later. They looked at postnatal day 21 instead of postnatal day 14. They also saw, they saw this, the selective loss of sodium current in the bipolar neurons, but now they also saw an increase in sodium current in the pyramidal neurons or the excitatory neurons. They saw both. Then when they looked at the excitatory neurons, they were spontaneously hyper excitable.

Dr. Lori Isom: 11:23 Later on, our colleagues led by Ethan Goldberg's laboratory saw something different. They looked at development of the mouse model. They looked at firing of inhibitory neurons as the mouse developed. They looked early on, I have to see if this works, oops, okay, at postnatal day 10, so way early before any seizures started. You can see that there's no difference in the firing of the inhibitory neurons. These are parvalbumin positive, inhibit, fast spiking interneurons. They saw no difference. You can see the quantification here.

Dr. Lori Isom: 12:05 When they looked at this mid range P18 or so, remember Bill Catterall showed it around P14, they saw the selective, they saw hypo excitability of parvalbumin positive, fast spiking interneurons at this age, okay, in the heterozygote, in that disease model, but not in the wild type. Then when they waited a little bit longer for the mice to become adults, around P35,

they no longer were able to see this difference. They proposed that there's a transient developmental window of fast spiking interneuron dysfunction in this mouse model of Dravet, okay? It becomes more and more complex.

Dr. Lori Isom: 12:48 Then to add even further complexity, De Stasi et al., in 2016 showed in a similar mouse model that they could, they saw this loss of firing in the interneurons. They looked in the parvalbumin positive and the somatostatin positive. They saw this as hypo excitability in the brain slice, so they agreed with the previous investigators. But when they use two photon imaging to look at the firing of either parvalbumin or somatostatin positive interneurons in the freely moving mouse, in a live mouse, they weren't able to see any significant differences between the wild type and the disease model.

Dr. Lori Isom: 13:36 What does this mean? It means that, oh, and before I go on, when Jack Parent and I first started looking at induced pluripotent STEM cell models of Dravet Syndrome, we saw something different. This work was done by Luis Lopez Santiago and Yukun Yuan in my laboratory. These were neurons made by Jack Parent's laboratory from Dravet syndrome patients and healthy controls. You can see some of the pictures here of a typical pyramidal neuron that Jack's lab made for us, and then a bipolar neuron or a GABAergic neuron. We measured sodium currents from both of these neurons. You can see the downward deflection by convention is an inward current and the current activates and inactivates. We saw when we quantified all the lines from two different patients compared to three different controls that we saw an increase in sodium current density in both the pyramidal neurons and the bipolar neurons.

Dr. Lori Isom: 14:38 On top of that, we saw that they were hyper excitable, that these are the Dravet versus the control. The take home message was that they were spontaneously hyper excitable, so they fired all by themselves in the dish as compared to the control neurons, which did not have this property, or we were really looking here at epilepsy in a dish. Now, this is different than what was shown in many of the mouse models.

Dr. Lori Isom: 15:05 So why am I showing you all this? Not to confuse you, but to tell you that it's confusing and it's complex. Using the models, the mouse models and the induced pluripotent STEM cell models, we and others see neurons subtype specific effects on sodium current and excitability in interneurons versus pyramidal neurons, so excitable versus, excitatory versus inhibitory neurons. These changes in excitability depend on the age or

neuronal maturity, so it's different in the neonatal versus the adult, or maybe the maturity of the IPS neurons. There may be some methods specific differences, so how are we measuring this? What are we modeling? Do we see different things in the brain slice versus in vivo recordings? Finally, is this model specific? Do we see differences in the mouse models versus the human models? Because as we've been known to say, mice are not small humans.

Dr. Lori Isom: 16:03 In fact, a few years ago, I wrote a commentary for epilepsy currents proposing that could everyone be right? Could all of these seemingly opposing models be modeling different aspects of a very complex disease? I'm telling you all this to tell you that there are many different targets of Dravet, there are many different neurons and neuron types that are changed. It changes with development. It changes in what model you're looking, and so we need a better therapeutic strategy.

Dr. Lori Isom: 16:41 In spite of recent small molecules, we don't know if fenfluramine and others are really going to have impact, but we decided in collaboration with Stoke Therapeutics to do something different. Instead of developing a small molecule to treat a disease that had already developed, to get at the source and to target the gene and the deficit there, so we could stop the progression of the disease early. Okay, woo, okay.

Dr. Lori Isom: 17:15 And so our colleagues at Stoke developed something called TANGO, targeted augmentation of nuclear gene output. The idea here, which is quite innovative, is to target the haplo-insufficiency of SCN1A and treat the genes, so that we can go from 50% of productive mRNA as Dravet patients have to 100% and hopefully increase the amount of protein made from half to the wild type 100% level.

Dr. Lori Isom: 17:56 How do you do that? SCN1A is the gene is composed of exons, that are coding for the mRNA protein, and then introns that are non-coding regions that have lots and lots of important regulatory information. SCN1A has at least one piece of regulatory information that is a nonsense-mediated decay exon, okay? What does that mean? When the gene is transcribed, we know if the nonsense-mediated decay exon is included in the transcript, then this encodes a premature stop codon, and this entire process enters nonsense-mediated decay, so it's gone. Alternatively, if this nonsense-mediated decay is spliced out or skipped, then the mRNA is productive and generates full length, functional protein.

Dr. Lori Isom:	19:06	This NMD exon is actually used in biology. It's used in the brain to regulate the amount of SCN1A that is normally produced or to turn it off in cells that shouldn't have it or to turn on more in cells that it should. Our colleagues at Stoke Therapeutics decided to harness this naturally occurring event. They developed antisense oligonucleotides or ASOs that specifically target this nonsense-mediated decay exon in SCN1A and prevent it from being expressed.
Dr. Lori Isom:	19:42	When it skips, when this is skipped and prevented from being expressed, this arm of the nonsense-mediated decay is prevented. It shunts all of the transcripts up into the productive mRNA and then the protein production, okay. We can amplify the amount of productive SCN1A transcript and then protein using this ASO technique.
Dr. Lori Isom:	20:12	This summarizes, this very nice figure summarizes from Stoke how this works. In the gene, in the cell there are two copies of each gene and you can imagine there's two copies of SCN1A. Without the TANGO ASO, if the nonsense-mediated decay exon is expressed, then half of the mRNA is shunted for degradation and only half then is transcribed and translated into a productive protein. But if the ASO sits on the nonsense-mediated decay exon and prevents its expression, then both alleles are able to go all the way through with productive mRNA and protein expression, okay? That's the theory. That's the mechanism.
Dr. Lori Isom:	21:15	At Stoke, they used a bioinformatics approach to start searching for these nonsense-mediated decay exons. They identified one. They showed that it was developmentally regulated. You can see a postnatal day zero through P20 and then onto 10 months, in the mouse, there are varying levels of expression of the transcript with the nonsense-mediated decay exon included or not, okay? The one on top is the one with the NMD. The one on the bottom is the one with not, and that's productive. You can see that when they quantified it here in the normal brain development of a mouse that early on both are expressed, and there's not very much productive SCN1A being made. Then as development ensues you see this increasing expression into adulthood. Interestingly, postnatal day, say around 16 or 17 here, when this becomes the highest is really the onset of disease in the mouse model.
Dr. Lori Isom:	22:25	When they looked at to see if this was conserved, not only in the mice, but in other species, they found that it was conserved in the rat, in nonhuman primates, in human brain, and as well as in the brains of Dravet patients. They, at Stoke, developed a

number. You can see 47 different potential ASOs here. They tested them. They tested them to look at two things, inclusion of this exon, okay, the nonsense-mediated decay exon, which they named 20N and production of full length transcripts. They wanted an ASO candidate that reduced the NMD exon inclusion to the maximum amount and increased the production of SCN1A mRNA to the maximal amount.

Dr. Lori Isom: 23:28 They found one, and that was ASO22. You can see it here with the red arrows, okay? They used it in cells. They could see that they could either get it inside the cell with free uptake, or they could nucleofect it in and actually get more. In a cell line, a human neuroblastoma cell line, they learned that it was pretty specific because this was important. They saw with it, treatment of the cell line, a selective up-regulation of SCN1A with no effects on other sodium channel genes that were present. That was important.

Dr. Lori Isom: 24:08 Okay, there we go. Then we tried it in mouse, because we need to know what happens in vivo. In this experiment, wild type mice, C57 black 6J mice were injected ICV, intracranial, injection at postnatal day two with a single dose. Then they were euthanized at postnatal day seven to look at what was happening in the brain. They measured non-productive SCN1A transcript or the transcript that included the NMD exon, productive SCN1A transcript that didn't include the exon, and the important take home message, Nav1.1 protein. They saw a dose dependent decrease in the amount of the nonproductive transcript, a dose dependent increase in the productive transcripts, and importantly, a dose dependent increase in protein.

Dr. Lori Isom: 25:08 This is a more extensive specificity study of all of the sodium channel genes that are expressed in humans. Again, you can see that this dose dependent increase, the full change in mRNA production was specific to SCN1A, you can see that in the black bars, but not to any of these other sodium channel protein, sodium channels genes. That was critical, because we wanted to target one gene and not others that may also be involved in epileptic encephalopathy. Then they use this to see how durable it was. They did an ICV injection at P2. Then they euthanized and collected tissue all the way out to postnatal day 32 and saw this dose dependent increase in SCN1A transcript and Nav1.1 protein. Now we were ready to go forward. We had a candidate, ASO22. We were ready to go in a disease model.

Dr. Lori Isom: 26:11 To summarize for everyone up to this point, this candidate ASO targeted a nonsense-mediated decay exon in the gene SCN1A in

both mice and humans, okay? This was conserved in both species. The effects on SCN1A mRNA and protein were shown to be dose dependent. The effects were specific to the gene SCN1A and did not affect other sodium channel genes.

Dr. Lori Isom: 26:39 At this point, we're ready to go in a mouse model. The mouse model we chose was one that was developed by our colleague, Dr. Jennifer Kearney at Northwestern University. It's a knockout model of SCN1A in a mouse. This time it targeted exon one, so the first exon of SCN1A transcript was deleted, okay? There was no chance of making any transcript or any protein from that allele, and so the heterozygotes, the plus minus, made half of the transcript and half of the protein. You can see half of the protein here. Here's a wild type mouse protein with Nav1.1 and half is made in the HAT, so you can see this very nice span at 250 kilodaltons. These mice have spontaneous seizures, sudden death. They're a SUDEP model. They've been shown to have cognitive and behavioral deficits in their robust model of Dravet.

Dr. Lori Isom: 27:41 I'm going to show this movie here, if this is a, this is a trigger, if anyone would not like to watch a mouse seize, oh, let's go backwards. Oops, darn. There it is. Okay, there's the warning. These mice begin to seize spontaneously. The earliest we've seen them seizes around P14. Normally they start to seize around P20, P21. They have a spontaneous SUDEP. You can see these seizures are quite violent, they're generalized. What happens at the end, you can see that hind limb flexion, and then the mouse, that mouse died of SUDEP. About 50%, 50% to 60% of the mice in this model have this phenotype. It was a very reliable model with a very big Y axis.

Dr. Lori Isom: 28:43 We tried this in my laboratory. We started out with this model. We injected cohorts of mice, so postnatal day two with a single dose of the ASO22. We chose 20 micrograms based on our dose response curves that were done previously. Then we began monitoring them. We monitored everybody by video recording under infrared light, beginning at postnatal day 17. We euthanized one cohort at postnatal day seven, I'm sorry, at seven weeks. We euthanized a second cohort at 14 weeks. This was dramatic.

Dr. Lori Isom: 29:21 This was, we did it blinded, but pretty soon it was pretty obvious which was which. The black line is a PBS control in the wild type mice. The red is PBS injected ICV into the SCN1A DS mice. We saw this robust SUDEP of, gosh, 60 or more percent of the mice by postnatal day 30 or so, okay? The most incredible result was that a single dose of ASO22 in these mice, in the DS

mice, saved every mouse except one. Actually, the day we lost one mouse, I thought good, because it was a little bit, it was just gray. It was too good to be true. We had one mouse, and so we had some biological variability. This was remarkable.

Dr. Lori Isom: 30:18 We collected tissue. We sent it to Stoke. They quantified the level of ASO22 exposure in the brains at seven weeks and 14 weeks. They looked at the full change in gene expression at seven weeks and 14 weeks. Then they looked at the full change in protein at seven weeks and 14 weeks. The great thing was that in the DS model, we went from half to a 100% in both times.

Dr. Lori Isom: 30:50 Now there was one thing that initially bothered me. As a sodium channel biologist, I was worried that we also got this dramatic increase in SCN1A mRNA and Nav1.1 protein in the wild type mice, so these guys here. I was worried that too much sodium channel may not be good either, but when we went ahead, when we went through the entire experiment and did not see any toxicity in the wild type mice that were treated with the ASO, that was very reassuring. Even though this additional protein is being made, perhaps it is not getting targeted, so the plasma membrane is not functional. Regardless, it does not cause toxicity in these mice. I'll show you further evidence of that as we go forward.

Dr. Lori Isom: 31:42 We also wanted to know what happens as we get closer to the time of disease onset. In these mice, the first, we in our hands, the first seizure we ever saw was right around P14, although usually it's a couple of days later. We decided to inject the mice then ICV at P14, right around the time when we saw the disease onset to see how close we could get and still have an effect of the ASO. We injected. We decided to go with 60 microgram dose, because the mice are bigger. We needed bigger brain coverage, so we went with three times the dose. We did the same groups. What we saw in both, and we euthanized here at P35 and P90, so we went all the way out to P90. We saw the similar increases in SCN1A mRNA and protein. We were able to positively effect a majority cohort of the mice, but not all of them. It was not as effective at this later dose as it was as the original.

Dr. Lori Isom: 32:55 Okay, so to take home to here, a single ICV injection of the ASO20 to reduce SUDEP incidents and increased Nav1.1 protein expression in our mouse model of Dravet. We were able to follow that all the way out to postnatal day 90, where we ended the experiment. We actually don't know how long it would have lasted. The ASO22 treatment also increased the Nav1.1 protein

in wild type mice, but did not result in toxicity. I'll show you that as assessed by a reduced survival, but we also did additional experiments to make sure that that was true.

Dr. Lori Isom: 33:35 The next thing we did was have our mice instrumented with EEG under video EEG in order to look at electrographic seizures, because there was the possibility that even though we weren't seeing the behavioral tonic-clonic seizures, that there could be subclinical electrographic seizures. We instrumented the mice or sorry, we did a single injection of the ASO22 at postnatal day two. This was the 20 microgram injection, so again, a single dose. Then the surgery was done at postnatal day 20 to instrument the mice and implant the electrodes. Then there was a short recovery period where we started the continuous EEG recording from postnatal day 22 to 46. You can see that here.

Dr. Lori Isom: 34:22 The number of seizures in panel B was significantly reduced from mice that were just injected with [vehicle 00:34:30] or PBS versus the mice that were injected with ASO22. The latency to first seizure, so they still did have some seizures, but the latency to first seizure was significantly delayed. Here's some typical electroencephalograms of wild type mice that then of course, we didn't see any abnormalities there. Then here, you can see DS mouse that was injected with vehicle just PBS, and they had a generalized seizure. Now it's interesting that some of the mice, we did see seizures in some of the ASO treated mice and that the seizures were not different than the seizure that we would see in the PBS treated mouse.

Dr. Lori Isom: 35:19 Okay, and not shown here is that mice injected with, the wild type mice that were injected with ASO22, remember that increased the mRNA and protein, they didn't die, but they also didn't exhibit any abnormal EEG activity during this recording period. Not only did we not effect their lifespan, we did not see any spontaneous behavioral seizures, but we also didn't see any subclinical seizures on the EEG.

Dr. Lori Isom: 35:50 Okay, so then we did, this is my student [inaudible 00:35:58], watched mice forever until he was ready to faint with video monitoring of behavioral tonic-clonic seizures in mouse cohorts. We put these under video monitoring. We did 24/7 recording. Then he studied the videos offline. He used software, but then he did it by himself by eye to find the seizures. He injected cohorts of mice, one with a 20 microgram dose of ASO22 at P2, one with this 60 microgram dose of ASO22 at P14, so right about the time of disease onset. Then he also did, because we

don't have that many containers for mice for my set with PBS at P14 to monitor. We monitored from P17 to P35.

Dr. Lori Isom: 36:52 The results was, I'll show you some examples, is that we saw a single tonic-clonic seizure followed by SUDEP in two of the four PBS injected mice. Just like I showed you in the video before, they would have that big seizure followed by SUDEP. In the mice that were injected at P2 with 20 micrograms of ASO22, we saw that event in one of 19 mice. 18 mice had no seizures, and they had no premature death, and we saw that seizure events in one. Then in the P14 injected mice we saw two that underwent that behavioral tonic-clonic seizure followed by SUDEP. The rest of the mice were unaffected.

Dr. Lori Isom: 37:46 Again if this trigger warning, I'm going to show some videos of mice having a seizure. These mice were injected with PBS on the left, okay? This is typical. This is like I showed you from Jennifer's mice. Spontaneous behavioral tonic-clonic seizure followed by, well, this mouse is in the mouse pile here, but continues to seize and then had the hind limb flexion and followed by SUDEP. If we watch it long enough, we can see that.

Dr. Lori Isom: 38:26 Now these mice over here, I need to increase this a little bit because they were sleeping for a minute, but okay, so this mouse was injected with the ASO. This is one of the two mice that we observed have the behavioral seizure followed by SUDEP. I want to show you this to illustrate the similarities between the phenotype. Okay, let's give it a minute. There's some stereotypic grooming behavior here, some jumping. You see the tail go up. Sorry, if there's a, I think there's a little bit of an internet slowdown, but there we go. The mouse has that same behavioral tonic-clonic seizure followed by hind limb flexion and SUDEP.

Dr. Lori Isom: 39:35 Well, okay. There we go. Administration of the ASO at P2 or P14 reduced the incidents but not the severity of the generalized tonic-clonic seizures in the DS mice. When a seizure was observed in the treated mouse, the morphology of the seizure appeared similar on the EEG and on the video to the PBS treated Dravet Syndrome mice. Although the incidents of the behavioral tonic-clonic seizures were dramatically reduced in the ASO treated Dravet mice, there were some subclinical electrographic seizures that we could detect by EEG and importantly, the wild type mice that were injected with ASO22 at P2 did not exhibit abnormal EEG activity during the recording period and did not have any behavioral abnormalities or early deaths.

Dr. Lori Isom: 40:36 Oh, okay. Let's recap our work to this point. It's preclinical evidence now shows that the ICV administration of a TANGO ASO designed to selectively prevent the inclusion of a naturally occurring NMD exon and human and mouse SCN1A transcript, so it works well in human and mouse, can restore productive SCN1A mRNA and Nav1.1 protein expression in Dravet Syndrome mouse brains up to wild-type levels, okay? The effect of ASO22 is remarkably specific to SCN1A and does not alter the expression of any other sodium channel alpha subunit genes either in cultured cells or in vivo. The ASO22 administration reduces the seizure and SUDEP incidents in the SCN1A heterozygous mouse model of Dravet Syndrome with no detectable adverse effects on the EEG pattern, the generalized behavioral seizures or survival in similarly treated wild type litter mates in spite of increased expression of Nav1.1 in the brains of these animals. Remember, this is a mouse model, and mice are not small humans. And so it's important to think about that going forward.

Dr. Lori Isom: 41:55 Oh gosh. Okay, so our colleagues at Stoke Therapeutics went ahead and did nonhuman primate study with ASO22 to ask whether or not, first of all, whether or not there's toxicity. They did single and multiple doses, and GLP stands for good laboratory practice. This time, they did intrathecal instead of ICV injection in nonhuman primates. Their key findings were no observed adverse effects at the highest dose treated, okay? In fact, this highest dose, the dose that they used here was the highest human equivalent dose that was planned to be administered in the clinical trial. They tried to exceed the doses that would be used clinically. They showed no changes in platelet counts or renal or hepatic function. They had no adverse histopathology in the brain and the spinal cord, the liver or the kidney. It appeared to be quite safe in nonhuman primates.

Dr. Lori Isom: 43:11 Then they looked at the distribution of the ASO, which is now named STK-001 in the brain of nonhuman primates. You can see that there was exposure in all brain regions except the pons and the thalamus, interesting. When they looked at Nav1.1 protein levels in these brain regions, they saw significant increases in the cortex, in the motor cortex and the prefrontal cortex with trends in other parts of the cortex. It looked good.

Dr. Lori Isom: 43:49 They also conducted something called the Butterfly Study, an observational study of Dravet Syndrome patients, that they conducted for two years of children and adolescents aged two to 18 to evaluate seizure frequency and non seizure comorbidities associated with Dravet. They looked at

intellectual disability, developmental disabilities, motor impairment, speech impairment, behavioral problems, and sleep abnormalities.

Dr. Lori Isom: 44:17 Happily the clinical trial was launched this past summer. It's called the Monarch Study. It was first in humans in August of 2020. The plan is to enroll 48 patients at about 20 sites in the United States with primary end points of safety and tolerability and with a single dose and look at the pharmacokinetics with secondary end points and changes in seizure frequency over 12 weeks and changes in quality of life. It's a two-part design. With a single dose in the first part of the design, and then later on to have increasing doses up to 30 milligrams for three months. For each dose level, there's a sentinel group of older patients, 13 to 18, followed by groups that are younger, two to 12. Hopefully we'll have preliminary data in 2021.

Dr. Lori Isom: 45:18 To summarize what I've told you today, this work demonstrates target engagement, pharmacology, and efficacy using the TANGO approach to selectively increase SCN1A gene and protein expression in a DS mouse model and in nonhuman primates. The strategy shows promise for ASO therapy to be a precise disease modifying treatment for Dravet patients and warrants preclinical testing to further evaluate safety and efficacy, which is ongoing. The TANGO approach is predicted to provide therapeutic benefit to a wide range of individuals with SCN1A variance that results in Nav1.1 haplo-insufficiency. What that means is truncating nonsense or frameshift variance or partial or whole gene deletions.

Dr. Lori Isom: 46:08 It's important to note here, the TANGO is contraindicated for patients with other kinds of STN1A variants that are more rare. I'm talking about the missense variants that do result in the generation of Nav1.1 protein that may have maladaptive gain of function or dominant negative effects, because what TANGO would do was also increased that protein expression, and in so doing could increase the disease severity. For these clinical trials, it's very, very important that patients are genetically screened, and those with known maladaptive gain of function or dominant negative variants are excluded. Finally, this work resulted in the launch of the Monarch clinical trial.

Dr. Lori Isom: 46:56 These are our collaborators at Stoke Therapeutics and especially the people that work directly with us, especially Zhou Han, Gene Liau, and Anne Christiansen who were the primary collaborators on the paper. Then this is my laboratory. Here, our funding sources, [Chun Lee Chen 00:47:16] and [inaudible 00:47:18] did the majority of the work on this particular paper.

I'd like to acknowledge my long-term collaborator, Jack Parent, with whom we do all of our induced pluripotent STEM cell work. With that, I'd like to thank you for attending today. I'm ready to take some questions.

Priya Balasubramanian: 47:37 Thanks so much, Lori. That was very interesting. It's so great to see that the clinical trial is ongoing. We have had some questions come in. Let's get right to them. If others have questions, you can type them in the Q&A box, and we'll try to get to them. The first question is about whether anything is known about the mutation susceptibility of SCN1A.

Dr. Lori Isom: 48:05 Can you say that again?

Priya Balasubramanian: 48:06 Is anything known about the mutation susceptibility of SCN1A?

Dr. Lori Isom: 48:11 Oh, you mean how susceptible the gene is to mutation? Very, very, very susceptible. It's a huge hit. It's a huge target for hits. We knew that back in the day when I was in Bill Catterall's lab and we were purifying and cloning sodium channels and cloning the SCN1A cDNA was just nearly impossible. Everything you did made it mutate. When we saw this later on, we were not surprised at all.

Priya Balasubramanian: 48:41 Awesome. Thank you. Then there's a question about where are you measuring expression levels in whole brain tissue or are you looking at specific brain regions?

Dr. Lori Isom: 48:54 Yeah, we get, so what we do is we take the whole brain and then take a slice that goes through the cortex and the hippocampus and use that to make the mRNA and protein.

Priya Balasubramanian: 49:08 Are you able to targets cell specific populations with ASO22? For example, inhibitory cells versus pyramidal neurons to see whether the therapeutic effect may increase?

Dr. Lori Isom: 49:24 Yeah, so this technique, as it stands, is general, so it does not discriminate between cell type. In fact, it will hit every kind of cell in the brain, including non neurons, okay? It seems to have this remarkable effect in spite of that. Now there are and encoded another biotech firm who was looking at this very similar technique but using viruses that target to PV positive interneurons. You may have seen their work at the AAS meeting last year. They have a very similar result with the SUDEP model that we have of stopping, of ceasing, of preventing the seizures and SUDEP.

Priya Balasubramanian:	50:14	Thank you. There's a question about quantification of the protein. Are you using Western blots to do that or are you doing something else?
Dr. Lori Isom:	50:24	Yeah, we're using the Western blots. It's more of a high throughput, so we've done it both ways. When you look in the paper, we've shown it by Western, which is more low throughput, but for all those millions of samples, we used a more high throughput protein, a high throughput modified method.
Priya Balasubramanian:	50:45	Let's see, there's a lot of questions here. We'll try to get a few more. There's a couple questions that came in about age of administration, especially since you're talking about P2 versus P14. How did that translate to humans?
Dr. Lori Isom:	51:03	That's a really good question. This is where mouse models have limitations. If you ask 10 developmental biologists, what a P2 mouse translates to in terms of a human, you will probably get 10 different answers whether or not that is a newborn, whether or not that's embryonic or not, and whether or not P21, at weaning, is the equivalent of one year. I've read some papers where that's more the equivalent of an older individual. It's really difficult to predict what the mouse ages are going to translate to with humans. That's why we wanted to push this up to the time of disease onset to see how much we could prevent. One of the limitations of this model is that there's such a small window between the time of disease onset and the majority of the SUDEP, so we start losing animals right around P22 to P23. There's very little window for us to do the injection and then have the full analysis before the animals start dying. I think that's where we've pushed this particular mouse model to the limit. That's why we've now doing the ultimate experiment, which is the clinical trial.
Priya Balasubramanian:	52:24	Have you looked at cognitive deficits in your mouse model?
Dr. Lori Isom:	52:28	We haven't, and that's a really good question. I get this question all the time. That's going to be a very, very large study. At present, we're looking at more detail at the electrophysiological details. We're going in and looking at sodium current and firing of the inhibitory neurons and whether or not we can reverse the disinhibition that we see. Once we get that nailed down, then perhaps we could go on and do some of the behavioral deficits as well.

Priya Balasubramanian:	53:02	You did mention that no other sodium channel genes are affected by ASO22 treatment, but did you look at the entire transcript on?
Dr. Lori Isom:	53:15	We didn't, and that's a good question. No, but that's something that we ought to do. Yes.
Priya Balasubramanian:	53:26	Do you think that you might need multiple injections to maintain the effect or is the single injection going to be enough?
Dr. Lori Isom:	53:34	That's a really good question that I get asked all the time. Based on our observation that we had a single injection at postnatal day two, and we ended our experiment 90 days later, and we still did not, we saw a single seizure and a SUDEP. The question is there may be two possibilities. Well, maybe three. Okay, so two possibilities that I can think of is that in a mouse, at least, the ASO gets the brain past a critical period of development with the increase in Nav1.1, and then after that, the brain takes over and plasticity happens and that it's normal development ensues, or this is a really long lasting ASO. We know that, that this chemistry really protects the ASO from degradation, so when you look all the way out at P90, you can see the ASO is still there. There's still some there. It may be that you need very little over a long time, and it's, it's very effective, okay, or maybe mice are not small humans, and there's a different process there, but at least in our hands, all you need is one.
Dr. Lori Isom:	54:48	Now in the, you notice in the clinical trial, they have two phases, right. They're doing a single dose, and then they're doing multiple doses to see what effect that is. If you think about the original ASOs news [inaudible 00:55:04], right, for spinal muscular atrophy, you have to do multiple doses. I think they inject quarterly, I think it is, in intrathecally in order to keep that therapeutic benefits, so that may be true.
Priya Balasubramanian:	55:20	Have the preclinical tests been performed in other mouse models? If yes, which ones?
Dr. Lori Isom:	55:27	They haven't. I think it would be an interesting experiment to do the test in one, in a model that actually makes a protein, okay? The models, most of the models that are out there now are haplo-insufficient. This was a null, but you could also do it in a, Dr. Yamakawa has a very interesting mouse model that has a knock-in of stop code on, okay, which is a humans variant. That would be interesting to do. Now, that's also haplo-insufficient, so we'd expect to have the same result as we have here. It would be interesting to prove the, what I proposed that if you

use a variant that actually makes a protein, that it also increases that protein and can have some deleterious effects. We should do that, but the only mouse model that we've used so far is the null.

Priya Balasubramanian: 56:28 What proportion of patients with Dravet would you say are good candidates for this therapy?

Dr. Lori Isom: 56:34 I think at least 60%. More than half of Dravet patients have variants that cause haplo-insufficiency. And so the nice thing about this ASO is it's a very antagnostic, right? Because so all of those variants cause nonsense-mediated decay of that other allele and this ASL upregulates the wild type allele, which is wonderful. I think at least 60% of the, and maybe more of the, maybe as close as 80% of the Dravet patients may be helped by this therapeutic.

Priya Balasubramanian: 57:10 Thank you so much. We'll do one last question. What is known about the location and type of SCN1A mutation related to the efficacy of TANGO in increasing protein production?

Dr. Lori Isom: 57:24 The location. Can you repeat that please, Priya?

Priya Balasubramanian: 57:28 What do we know about the location and the type of SCN1A patient and how is that related to how effective TANGO might be in [crosstalk 00:57:37]?

Dr. Lori Isom: 57:37 Yeah, totally agnostic. It doesn't matter. If the mutation or the variant causes a premature stop or a deletion and targets that mRNA for nonsense-mediated decay, then this ASO will work. It doesn't matter where. On that map that I showed you that you could have a mutation at the end terminus or the C terminus and still have Dravet due to haplo-insufficiency, the ASL will work with all of those, as long as it causes haplo-insufficiency. That's the beauty of this treatment that it doesn't matter where the variant is.

Priya Balasubramanian: 58:18 Very interesting. With that thank you so much, Lori. I'd like to conclude this virtual seminar. Again, thank you very much for your presentation. I'd also like to thank the Nussenbaum-Vogelstein family for supporting this Frontiers in Epilepsy Research Seminar Series. I'd like to thank everyone in the audience for all the great questions and for staying engaged. If you'd like more information, you can always visit our for researchers page on our website, or you can also email us at research@cureepilepsy.org. We do plan to continue these in 2021, so please check back in early 2021. We do really value

your feedback, so we'd really appreciate if you could take the brief survey that will follow at the end of this seminar. Thank you all once again and stay safe.