



CURE Epilepsy Seminar
Biological Mechanisms of SUDEP
Lecturer: Dr. Kristina Simeone
(Transcript)

- Priya Balasubramanian: [00:00](#) Welcome, everyone. I'm Priya Balasubramanian. I'm associate director of Research at CURE Epilepsy. I want to thank you all for joining us today. Today, we bring you our second virtual seminar. This is part four, 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 do exciting epilepsy research. It also provides opportunities for young investigators to interact with leaders in the field. Because of the current 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 in person, we will present these seminars virtually.
- Priya Balasubramanian: [00:49](#) As we approach SUDEP Action Day this Friday, today's virtual seminar is entitled Biological Mechanisms of SUDEP. It will broadly discuss the cardio-respiratory dysfunction associated with SUDEP, identification of biomarkers to potentially better-defined risk for SUDEP, and the role of the neuropeptide orexin as a central regulator of pathways that can contribute [inaudible 00:01:14]. CURE has been proud to be a leader in the epilepsy research community for over 20 years, funding over 240 projects spanning 15 countries. We currently have three different funding mechanisms. Our key research priority areas include acquired and pediatric epilepsy, treatment-resistant epilepsy, sleep and epilepsy, and Sudden Unexpected Death in Epilepsy or SUDEP.
- Priya Balasubramanian: [01:41](#) All of our grant applications progress through a letter of intent phase and then a full proposal review phase reviewed by scientific reviewers as well as members of our community who are touched by epilepsy. The CURE Epilepsy Award is open to established investigators, whereas the Taking Flight Award is intended to support junior researchers who have at least three years of postdoc experience but have yet to obtain significant funding. You can look up for the call for these two awards, the CURE Epilepsy in the Taking Flight awards, which will be coming out later this year. Our third mechanism, the Catalyst Award, is our newest award and is intended to fund translational research that aims to advance new therapies and clinical application.
- Priya Balasubramanian: [02:27](#) Today's presenter is Dr. Kristina Simeone, Associate Professor in the Department of Pharmacology and Neuroscience and Director of the Master's in Neuroscience program at Creighton University School of Medicine. Dr. Simeone's primary research interests include understanding how metabolic and pharmacological therapies influence seizures and longevity in animal models of epilepsy and identifying mechanisms underlying SUDEP and understanding the complex relationship between sleep procedures. Dr. Simeone is a 2016 CURE Epilepsy grantee. Her grant which focused on identifying role of orexin in the pathophysiology of

SUDEP was generously funded by the Benninghoven Family in memory of Cameron Benninghoven.

- Priya Balasubramanian: [03:14](#) Before she begins, I would like to encourage everyone to ask questions. You may submit your questions any time during the presentation by typing them into the Q&A tab located on the bottom of your Zoom panel and click Send. We'll do our best to get through as many of the questions as we can. Finally, I want to mention that today's virtual seminar as well as all of our future seminars will be recorded and will be available on the CURE Epilepsy website. Now I'll turn it over to Kristina for her talk. Thank you.
- Dr. Kristina Simeone: [03:48](#) Good afternoon, everybody. Thank you so very much for the invitation to speak today and to share my research with you. I'm absolutely honored to be here speaking before you today. Today, I'm going to be sharing some of the research that's been generated in our lab on the Biological Mechanisms of SUDEP. You can see in the title, I put an S by SUDEP to indicate SUDEPS. I think in the field, we're all starting to realize that there might be multiple mechanisms that are upstream of SUDEP and that might be contributing to SUDEP. Whether or not these mechanisms individually or collectively contribute are things that we just really don't fully understand.
- Dr. Kristina Simeone: [04:31](#) So, today, I'll be sharing some of the data that we've generated as we've been in search for biomarkers that may better predict a temporal prediction of SUDEP. So, that is to say, not only just a risk factor, like generalized tonic-clonic seizures are risk factors, but this would be a temporal prediction. So, this would be something that changes within a person or within a preclinical subject that is temporarily associated with when SUDEP up is going to happen.
- Dr. Kristina Simeone: [05:01](#) So, today, we'll start by giving a brief background of just some of the risk factors associated with SUDEP and the terminal event itself from our clinical studies. And then I'll share some of our within subject biomarkers that we have found or within subject changes that we have found in our preclinical animal model. And then in the second half of the study, I'll talk about how orexin may be contributing to some of these.
- Dr. Kristina Simeone: [05:31](#) So, our overall hypothesis in our laboratory is that there are these progressive pathophysiological changes that happen that can increase susceptibility to SUDEP. So, we know that severe seizure types, the generalized tonic-clonic seizures are a risk factor for SUDEP. We know that when these seizures are refractory to traditional anti-seizure drugs, that that also can increase risk for SUDEP. In our preclinical animal model, it is the Kcna1-null model. So, the Kcna1 gene was knocked out. These animals do not have the Kv1.1 protein. The Kv1.1 protein makes the alpha subunit for the heteromeric delayed rectifier potassium channel, but it's a

heteromeric channel, so other subunits can compensate for the loss of the Kv1.1.

- Dr. Kristina Simeone: [06:31](#) So, some advantages of this model are that all of the animals developed spontaneous recurrent seizures. Interestingly, this makes some of the research a little bit difficult, but it makes it more clinically relevant. So, the animals have different seizure phenotypes. So, some of them may have fewer seizures. Some of them may have more. Some of them may have a lot of mild seizures. Some of them may have a lot of generalized tonic-clonic seizures. So, there really is a lot of variability in this population in this preclinical model, which I think is more clinically relevant.
- Dr. Kristina Simeone: [07:06](#) So here in the middle panel, I'm just showing that there are three EEG traces. The first one is showing a mild spike wave discharge that's associated with a myoclonic jerk. So, the EEG trace is in black. The time-frequency analysis is right above it. This is a more severe seizure type that our animals experience. So, this is a stage three seizure. So, we have more EEG activity. We have higher frequency oscillations occurring during that EEG activity, and that's associated with [inaudible 00:07:40] clonus. A stage five severe seizure is a generalized tonic-clonic seizure.
- Dr. Kristina Simeone: [07:46](#) Some other advantages of this model, so throughout this talk, I'll be calling them KO for knockout. So, the knockout animals are the ones that have spontaneous recurring seizures. So, they have epilepsy. So, another advantage of this model is that all of them die suddenly. We have generated a survival curve of our colony as shown here. So, this allows us to do a couple of things. One, we can look at endpoints throughout the entire life of the animal until they die and then look to see if there were any within subject changes right before they died.
- Dr. Kristina Simeone: [08:18](#) So, I'll be sharing some of that data with you today. In addition, if we have some experiments that are terminal, we've generated survival curves and we can describe the animals in terms of probability of sudden death. So, just like for pharmacology, you would do an EC50 or an IC50 for a dose. Here we do SD50s for the percent probability of sudden death. So, at an age of SD50, 50% of the colony has died. So, I'll be using that nomenclature in some of the slides during this talk.
- Dr. Kristina Simeone: [08:50](#) A few years ago, the MORTEMUS study was published by Ryvlin and colleagues. What this showed us for the first time was they were able to capture a SUDEP event in clinic in patients that were monitored with an EEG and respiratory monitoring and ECG monitoring, all simultaneously. What they found is that there was a generalized tonic-clonic seizure in all of these patients, followed by rapid breathing, followed by periods of apnea, which is shown in light blue for each of these patients.

- Dr. Kristina Simeone: [09:22](#) So, here, this graph is showing nine patients. The top bar with the blue colors are showing the respiration data. The second bar for each patient in a doublet series is showing purple and salmon color and red and that's showing heart rate changes. The x-axis is time. So, all of these patients at time zero, they had a severe generalized tonic clonic seizure, followed by rapid breathing, and then subsequent periods of apnea and bradycardia as shown by light blue and purple, prior to terminal apnea and asystole shown in dark blue and red.
- Dr. Kristina Simeone: [10:04](#) So, the endpoints that we looked in our preclinical model, we wanted to look at heart rate and see if they had any bradycardia. We wanted to look at respiration. We wanted to see how their blood oxygen saturation was, because we know that intermittent hypoxia can cause bradycardia. So, respiration, apnea, heart rate, and blood oxygen saturation. We looked at one other important, we looked at sleep, because if you're deficient in sleep, it can lead to a lot of these pathologies. So, those are the five endpoints I'm going to be sharing with you today.
- Dr. Kristina Simeone: [10:40](#) So, the first one here is intermittent bradycardia. So, what I'm showing is if you look at the first scatterplot, the first column of data is in blue. Underneath that, it says WT for Wild Type and this is just the heart rate. So, the first scatterplot is just showing you the normal variability in the control animals that we have for all of the ECG traces that we recorded. If you look at the second column, this is a scatterplot of all the ECGs of our epileptic population. You can see anything that is below this or in the salmon colored highlighted box is a bradycardia event or an ECG tries to have bradycardia.
- Dr. Kristina Simeone: [11:22](#) So, you can see that in our epileptic population, they're significantly more bradycardia. When we stratified the data by age, you could see that the younger ages. So, now, if you look at the x-axis, we're increasing in age as we go from left to right. You can see the younger animals look a lot like wild type. These are ages that have a low probability of sudden death. And then as you increase the probability of sudden death, you could see that the ECG traces are starting to have more bradycardia events in them.
- Dr. Kristina Simeone: [11:56](#) So, we know that this study was published just this year. We have many other figures. I just wanted to share with you this one to describe this intermittent bradycardia that we found in the high-risk epileptic population or the animals that were at high risk for sudden death. We also found that this was associated with an increase in HRV or heart rate variability. An increase in HRV is usually thought to be caused by an increase in parasympathetic drive. So, I'll show you a little bit of that autonomic data halfway through the talk. I have another slide that describes that a little bit more detail. So, we'll be coming back to this.
- Dr. Kristina Simeone: [12:39](#) So, the first kind of biomarker that we saw was this intermittent bradycardia. Now, we know that generalized tonic-clonic seizures can

cause hypoxia. We know that intermittent hypoxia and blood gas instability can cause bradycardia. So, next, we wanted to look at oxygen saturation. So, we used pulse oximetry. So, I just wanted to share with you some traces before I show you the quantification graph. So, here, we did find that in our epileptic mice, that there was intermittent hypoxia. So, these guys could be considered have chronic intermittent hypoxia. So, if you look at these traces, we're looking at A. So, A is just wild type in an epileptic pulse oximetry trace. The x-axis is time. So, we're looking at these traces over time.

- Dr. Kristina Simeone: [13:33](#) Wild type blood oxygen saturation is around 97. It's really stable. It's shown by the black line at the top, and it just stays pretty straight throughout the trace. If you look at the epileptic trace or blood oxygen saturation, it's in blue. You can see that the oxygen saturation is just dipping a lot. Anything below 90% here is considered hypoxic. So, you can see here that there are two dips, where it was considered hypoxic. Now if you go through Figures B through F, these are all knockout data.
- Dr. Kristina Simeone: [14:11](#) So, there's no wild type data here. This is just to show you that there were times in the knockouts where the blood gas stability as shown by the blue lines was absolutely fine. So, here we have a blue line showing blood gas stability during normal breathing, shown by the green line which is eupnea. We also saw a normal blood gas stability during a normal pulse. The blood gas stability is plotted in blue. The normal pulse is plotted in red. This is Figure C. And then also during a bradycardia event, you could see that we still had normal blood gas stability here during this bradycardia event.
- Dr. Kristina Simeone: [14:45](#) D, E and F are showing hypoxic events that can occur during seizures. So, here are two examples here, where hypoxia was occurring during the seizure. E is showing that you had a hypoxic event that preceded a bradyarrhythmia. F is showing that you had a hypoxic event that was occurring during eupnea. We also had hypoxia occurring during apnea as well, but that graph isn't shown here.
- Dr. Kristina Simeone: [15:12](#) So chronic intermittent hypoxia is another phenotype. Actually, let me show you one more graph here before I conclude. So, these are epileptic animals that were at SD90. So, 90% of the cohort had died by this age. These are aged matched wild type littermates. So, anytime we're looking at an epileptic animal, they always have a wild type littermate that's paired with them or they're yoked throughout the whole study. That's their age-matched control. So, at this age, you can see I have four columns of scatterplots. The first one and the third one are the wild type data. It is the oxygen saturation during periods of rest in the first column and during periods of activity during the third column. You can see that the wild type controls have normal blood oxygen saturation.

- Dr. Kristina Simeone: [16:00](#) But if you look at the epileptic, the SD90 epileptic animals, you can see that during periods of rest that their blood oxygen saturation is often hypoxic. If you look at periods of activity, you can also see that their blood oxygen saturation is often hypoxic. So, we know intermittent hypoxia is associated with early mortality in heart failure. These data would suggest that intermittent hypoxia becomes a problem prior to sudden death in the epileptic animals. I haven't shown you here are the younger animals, which their blood oxygen saturation looks a lot like wild type. They're pretty stable. So, this would indicate there's intermittent hypoxia in the older, higher risk epileptic animals, and that there's blood gas instability in these animals.
- Dr. Kristina Simeone: [16:56](#) So, we wanted to look at breathing. We've done a lot of different types of breathing tests. One thing that we found is when you challenge a wild type animal, it doesn't really change breathing a whole lot. But if you could challenge an epileptic animal, here, they will have this rapid breathing response where they increase their breathing rate. So, this population was SD55. So, at this age, half of the epileptic animals had already died a sudden death. We're going to come back to this in the second half of the talk. We're going to expand on this rapid breathing a little bit more.
- Dr. Kristina Simeone: [17:32](#) One thing that was noted in the Ryvlin MORTEMUS study was that the subjects prior to passing away of SUDEP, they had this generalized tonic-clonic seizure and then they had rapid breathing. That caused apnea. Now, typically, rapid breathing increases blood gas stability, and then that can stabilize behavior and arousal and waking up from anything that was a hypoxic and hypercapnic event or really severe seizure, for example.
- Dr. Kristina Simeone: [18:11](#) But in the Ryvlin study, that rapid breathing promoted apnea. In the literature, that's only seen in one other event where rapid breathing promotes apnea in central idiopathic apnea. So, people that have central idiopathic apnea, they have this rapid breathing induced apnea. So, we found that our epileptic guys during challenges, they would have this increase in rapid breathing the closer that they got to SUDEP or higher probability of sudden death.
- Dr. Kristina Simeone: [18:44](#) Here, what we're showing is when we looked at different ages, so SD20, so here 20% of the colony has died of sudden death. So, they're a low probability of sudden death. Through SD55, so three different groups here, SD35 and 55. We're looking at hypotony or shallow breathing, which is thought to be a phenotype that can occur prior to the onset of apnea. In individuals, we found that there was an increase in both hypopnea. By SD55, if you look at the graph on the right, 100% of subjects are experiencing apnea in our epileptic cohorts. So, we do have an increase in rapid breathing. We do have an increase in apnea. We have that increase in intermittent hypoxia and bradycardia.

- Dr. Kristina Simeone: [19:33](#) So, the last endpoint I wanted to go through with you in the beginning of this talk or the first half of this talk is sleep. So, right now, as a nation, partial chronic sleep deficiency is actually a public health problem right now. So, partial chronic sleep deficiency means that you're not getting enough sleep for one night, that's partial sleep deficiency. You're not getting enough sleep for one night. Chronic means this happens multiple nights in a row. Because of our lifestyles, I think most of us have partial chronic sleep deficiency or a lot of individuals do. It's definitely a public health crisis right now, in the United States.
- Dr. Kristina Simeone: [20:20](#) Now, in the non-epilepsy realms, sleep deficiency alone can cause cardiovascular problems. Partial chronic sleep deficiency is associated with respiratory problems. Partial chronic sleep deficiency is associated with apnea. It is associated with blood gas instability. It is associated with all of these endpoints that we saw were changing in our epileptic animals. So, we wanted to determine whether or not there was a sleep deficiency in our epileptic animals prior to them passing away of sudden death.
- Dr. Kristina Simeone: [20:54](#) So here, what we did is we looked at sleep every day of their life until they passed away or died suddenly. And then we plotted it retrospectively from the day of death backwards. What we found is when you look at the sleep deficiency that was occurring, it was chronic. There was some deficiency that was happening every night. So, they weren't getting enough sleep every night. So, this was a partial chronic sleep deficiency, and it was getting worse definitely 10 days out prior to death. But starting about two weeks out prior to death, they were having significant sleep deficiency problems. So, those were the biological pathophysiology that we found.
- Dr. Kristina Simeone: [21:38](#) So, we use whole body, integrated systems biology approaches when we're looking at SUDEP, because a lot of these endpoints are very interdependent on each other and they can cause some of the other endpoints. So, whether or not there's a central event that's causing all of this or if different things are causing them or if they're interrelated, all of that still needs to really be tangled out or untangled. So, after we discovered some of these endpoints, this led our lab into two different directions. So, one direction was the SUDEP Survey, which I'll talk about for just a few minutes here. The other direction was more of a basic science approach, which was looking at orexin and seeing if the neuropeptide orexin was upstream of any of these changes.
- Dr. Kristina Simeone: [22:26](#) So first, I wanted to spend just a minute or two talking about this survey. So, two years ago, I presented some of this data, some of these changes within subject changes. I presented it at the PAME Conference in 2018. So, that's the Partners Against Mortality in Epilepsy. After my talk, several family members were eager to talk to me, because they had seen changes in their loved one prior to their loved one passing away of SUDEP. They

had seen some of the changes that I had presented in my talk. Interestingly, each person saw something different.

- Dr. Kristina Simeone: [23:06](#) One of the parents that was talking with me after my talk, she was telling me that her son noticed a change. He noticed a change himself. He was talking to her about these changes. She really didn't understand him and had to look them up online what they meant. And then he had passed away of SUDEP a few weeks later. So, my really good friend, [Dawn Martens 00:23:34], and colleague, she has a daughter that has Dravet syndrome. So, she's very in tune with her daughter's epilepsy.
- Dr. Kristina Simeone: [23:45](#) During one of the breaks at the PAME Conference, we were just chatting and decided that we really need to be able to capture and better understand insights that family members might have from the family members or loved ones of people that had passed away of SUDEP. So, we just finished writing the survey. It's IRB approved. We're going to put it out next week. The point of the survey is we're asking if they noticed any change to their loved one prior to them passing away of SUDEP or if their loved one notice a change and talk to them about it prior to their passing away.
- Dr. Kristina Simeone: [24:24](#) So, hopefully, next year, the SUDEP Survey is going to be available on the SUDEP Institute. Hopefully, next year, I'll have some responses to share with everybody. I'm going to distribute these findings with the SUDEP research community, that clinical and basic science research community, in hopes that some of the insights that they might be able to provide can really steer the future directions of how we're researching SUDEP. So, that was one avenue that our basic science studies had set us on. So, hopefully, next year, we'll have some more and really exciting information to share from the responses from that survey.
- Dr. Kristina Simeone: [25:05](#) Now, the second avenue was done in our laboratory. It was a basic science approach. So, it was looking to see whether or not the neuropeptide orexin could maybe be upstream of a couple of these endpoints that I just shared with you. So, orexin is a little protein neuropeptide, that's expressed in the lateral hypothalamus. It's in the middle here. It's shown in red on the schematic on the left. It projects to a bunch of different places. So, I made a second schematic over here on the right, just showing where all of the brain regions to which orexin neurons project. So, we know that orexin neurons project to heart rate or heart rate regulating brain regions and can promote both tachycardia and bradycardia, depending on where they're projecting.
- Dr. Kristina Simeone: [25:58](#) We know orexin neurons are upstream of respiration. They can control inspiratory time, breathing frequency, breathing volume, by projecting to all of these brain regions and directly projecting to the phrenic nucleus, which controls the phrenic nerve activity. Orexin also projects to arousal regions of the brain, which is responsible for waking you up after you've

been sleeping. That's how we originally got into the orexin literature was because we were looking at the sleep deficiency. So, when you're sleeping at night, orexin is activated, and it triggers all the wake promoting brain regions and it helps you stay awake throughout the day.

- Dr. Kristina Simeone: [26:41](#) All righty, so you can imagine orexin is not always involved in baseline when you're just sitting, heart rate and respiration. But during some kind of an event or a challenge, orexin is upstream of the cardio respiration. Depending on the situation, if you imagine it as a dial, it can increase or decrease the tone of the response of the cardio-respiratory response to two different types of challenges. We also noticed that we had some subdural EEGs and depth electrodes implanted in the lateral hypothalamus as shown on the top trace. The cortical EEG on the second trace, we found that the generalized tonic-clonic seizures propagated down to the lateral hypothalamus. The less severe seizures did not propagate down to the lateral hypothalamus, just the generalized tonic-clonic seizures.
- Dr. Kristina Simeone: [27:45](#) In addition, we knew that in other brain regions that experienced seizure activity, there could be differences in protein expression. There could be differences in cell number. So, we wanted to determine whether or not anything in the lateral hypothalamus had changed as a result from animals that had experienced generalized tonic-clonic seizures, in which the seizure had propagated down to the lateral hypothalamus. So, we did immunohistochemistry. We measured and we looked at orexin. So, on the left and Figure A, you can see orexin neurons in green. On the right, we're just quantifying these orexin neurons. Now, the blue bars are indicating your wild type levels. Orexin levels typically increase until they hit adult levels and then they stabilize.
- Dr. Kristina Simeone: [28:32](#) Now, these are age-matched wild types to our epileptic animals, which is in the second bar in the red hatched bar. We have two ages here that are SD0s. So, SD0 is prior to epilepsy onset and SD0 is at epilepsy onset. These two time points mean that they're at very low probability or zero probability of sudden death at these ages. And then SD10 and SD30. You can see at SD10, the epileptic animals or seizure onset has already started. Their orexin levels look similar to wild type. However, by SD30, there's like a 20 to 25% increase in the number of orexin neurons in epileptic animals.
- Dr. Kristina Simeone: [29:19](#) So, because we know if we look over here to the right and we look at orexin, we know orexin can project to heart rate regulating regions. It can specifically, if activated, promote bradycardia by acting through the nucleus tract solitarius, the cardio vehicle neurons, and the nucleus ambiguus. So, I wanted to go through this top graph that is highlighted in this really key lime green and then this bottom graph down here that has 'Atropine' at the title. But first, I want to go over to the far left to see the title of the slide.

- Dr. Kristina Simeone: [29:54](#) Here, what we found is blocking orexin receptors with a dual orexin receptor antagonist. So, there's two orexin receptors, orexin receptor one and two. The dual orexin receptor antagonist is a term that's used clinically to talk about these drugs. So, it's called a DORA. DORAs are FDA approved to treat sleep disorders. Again, if you're blocking the wake promoting signal, that's how it's inducing sleep. You don't have that next day drowsiness side effects that a lot of the benzodiazepine sleep aids have, and the benzodiazepine-like drugs have.
- Dr. Kristina Simeone: [30:27](#) So here, we found that blocking orexin receptors with a DORA was able to stabilize the heart rate. Now, I want to be very careful and go through this a little bit slowly. One point that I really want to make is in all of our studies and all of our endpoints, we really appreciate the nuances. We really take the time to go through all the data. It might be automated or semi-automated method, but everything is manually verified.
- Dr. Kristina Simeone: [30:55](#) We find for a lot of these endpoints, because the epileptic mice have variability in their seizure phenotype, there's usually a lot of variability in our epileptic populations. But we usually find that there's different populations. You can divide these out and stratify the data into responders and non-responders or ones with pathology, ones without pathology. So, I think for the SUDEP field, in order for us to really understand SUDEP, we're really going to have to appreciate and highlight these nuances and make sure we're stratifying the data and that we're looking at things very, very carefully.
- Dr. Kristina Simeone: [31:35](#) So, we did this in this study. Now, if you look at the top green graph, the one I wanted to go through first, this was a really big long graph. Heart rate was on the y-axis on the end. But it wasn't necessary to go through all of that, I just wanted to highlight this one section. So, I do apologize, my y-axis got cut off. But what I wanted to show you here, we have all of the red box-and-whisker bars, it's one epileptic animal. It's all the ECG traces that we recorded from that one epileptic animal. All right. And then the hatched bar right next to each red bar is going to be the same animal three days later when we recorded heart rate again. This was after the animal was treated with a DORA or we were blocking orexin receptors.
- Dr. Kristina Simeone: [32:19](#) Now, because you don't have heart rate on the y-axis, this blue line here is indicating bradycardia. So, anything lower than the blue line indicates that that animal had bradycardia or intermittent bradycardia. So, these first two animals you see did not have any bradycardia events. They were epileptic animals. They did not any bradycardia events. When you give them a DORA, it didn't really do much to their heart rate. Now, in wild types, when you give a wild type of control animal a DORA, it doesn't really do much to its heart rate either. So, these looked a lot like how a wild type would respond.

- Dr. Kristina Simeone: [32:50](#) In these six knockouts, these red bars, all of which went below the blue line. You can see three days later, a couple days later, when we gave these knockouts, a DORA, the dual orexin receptor antagonists, we were able to stabilize heart rate. Only one of them that had a little bit of bradycardia, but bradycardia was significantly reduced in every single one of these knockouts. Now bradycardia is usually associated with heart rate variability and with an increase in parasympathetic drive.
- Dr. Kristina Simeone: [33:23](#) So, one of the findings we found from the CURE-funded study was in addition to many findings I'm reporting and sharing with you today, one of them was that this response, this increase in bradycardia, this orexin-independent increase in bradycardia was dependent on orexin's actions through the parasympathetic system. So, blocking orexin receptors was able to stabilize heart rate and the animals that had bradycardia. This was through a parasympathetic mechanism. Alright, so that was the first one.
- Dr. Kristina Simeone: [33:57](#) All right. Next, we wanted to look at breathing rate. Remember, I talked about sometimes, the knockouts, when you challenge them with different kinds of challenges, they have an increased breathing rate. One of the challenges that we've done in our knockouts is a methacholine challenge. Typically, in a methacholine challenge, when you increase the doses for control wild type, the response is their breathing rate goes down. In the epileptic animals, their breathing rate can go up. Now here, what I'm showing you is let's look at the top right first, so we have orexin.
- Dr. Kristina Simeone: [34:35](#) Now, I wanted to highlight everything in blue, so orexin can project to all of the respiratory centers or all of the centers of the brain that control respiration. I wanted to share with you that when we blocked orexin receptors in the epileptic animals, that the breathing rate was stabilized to wild type levels. So, it was blocking orexin receptors, and the epileptic animals reduced the breathing rate. This is another within subject study, where we're exposing the animals to a methacholine challenge.
- Dr. Kristina Simeone: [35:13](#) So, in red was when the epileptic animals were treated with the vehicle. And then right next to it was a couple days later, they were treated with a DORA and exposed to the same challenge. You can see breathing rate was reduced at these lower doses of methacholine in all of the animals. So, this means that this increase in respiration for this particular challenge was mediated through orexin, in orexin's activity or hyperactivity in the breathing response.
- Dr. Kristina Simeone: [35:41](#) Now, if you go to 24 and 48 mgs per kg of methacholine, so more severe challenge, the increase in respiration is not orexin-independent. This is through another mechanism. Alright, so blocking orexin receptors was able to stabilize the breathing rates of the epileptic animals through lower dose methacholine challenges.

- Dr. Kristina Simeone: [36:04](#) All right. So, we have a couple more endpoints to go through. I think it's apnea, hypoxia, and sleep. So, here is apnea. So, we found that blocking of orexin receptors was able to decrease apnea. So, here again, we have three different ages, SD20, 35, and 55. This was the data I showed you earlier. So, by SD55, 100% of our epileptic animals experienced apnea. Now, interestingly, at SD20, some of the subjects experienced apnea, but it isn't dependent on orexin.
- Dr. Kristina Simeone: [36:43](#) However, by SD55, we see that 100% of the subjects are experiencing apnea. If you block orexin receptors, it protects a lot of these subjects from experiencing apnea. This is a within subject. So, these are the same animals, so protected them from experiencing an apnea. However, there still were some subjects that did experience apnea. So, it didn't get rid of apnea in all of them. So, we thought that was interesting and unexpected. We were not expecting any changes in the apnea. We can talk about that in the interpretation of that in just a few moments.
- Dr. Kristina Simeone: [37:22](#) So, next, I wanted to share the sleep data. So, we found that blocking orexin receptors did in fact improved sleep. So, here, I wanted to share the hypnograms, because sometimes, the hypnograms are more fun to look at than the bar graphs. So, here, we have a wild type control hypnogram on the top. So, let's just look at that one first. So, on the x-axis, we have time. So, it's a six-hour hypnogram. In the y-axis, you have wake, non-REM and REM. So, the state of vigilant that that subject is in at that particular moment in time. You go through the EEG, EMG, and video data. You do 10-second EPICs. You determine whether or not the animal is awake, in non-REM or in REM.
- Dr. Kristina Simeone: [38:07](#) So, what we found, after analyzing this is that the wild type animal spend quite a bit of time in non-REM and in REM. REM onset happens about an hour or so into the rest phase and then they experience non-REM and REM throughout the rest phase. If you look at a typical epileptic... So, this is our epileptic animal, Kcna1-null is the same as a knockout, which I was referring to earlier, the epileptic animal. ... they spend a lot more time awake, less time in non-REM and significantly less time in REM. One of the animals actually didn't have any REM at all. You can see that there's a huge increase in the latency to REM onset, it takes a long time for REM to even happen.
- Dr. Kristina Simeone: [38:54](#) Now earlier, I told you that orexin is upstream of wake promoting regions. That's why dual orexin receptor antagonists or DORAs are FDA-approved and new drugs that they're using for things like insomnia. Because if you block the wake signal, then you can help an individual fall asleep faster and prevent the sleep fragmentation or prevent them from waking up a lot in the middle of the night. So, we gave a DORA to our epileptic animals. We found there was a significant increase in non-REM and an increase in REM. Not only that, but REM latency to onset REM happened a lot faster. So, blocking orexin receptors did indeed improve

the sleep architecture of this epileptic population. These were specifically high risk within subject study.

- Dr. Kristina Simeone: [39:46](#) So, we did the hypnograms and the EEG, EMG analysis during a vehicle treatment and then also doing a direct treatment. So, we found that improves the architecture. We also found increased non-REM duration. So, when they were in non-REM, [inaudible 00:40:04] a bit longer. There was a faster onset of REM, which I showed you in the hypnogram. We also found and this was surprising that we had a reduction of seizures in this study. This was the incidence of some of the knockouts that were treated with a vehicle after they were treated with Almorexant, that there was a reduction in their seizures.
- Dr. Kristina Simeone: [40:27](#) Now, these were stage two through five seizures. If you look at the spike wave discharges, there was not a difference in the spike wave discharges. Over here in C, this is seizure burden. So, it's a way of accounting for both frequency and duration. So, it's a summative metric, accounting for the severity of seizure and the duration. So, seizure burden was greatly reduced in the animals that were treated with a dual orexin receptor antagonist.
- Dr. Kristina Simeone: [41:01](#) There was a very strong correlation between the animals that REM onset happened really quickly, they had the lowest seizure burden. If it took a really long time for REM to kick on, those animals had a greater seizure burden. So, this was a sleep study. We don't know it was very difficult to tease out whether the sleep was improving the seizures or reduced seizures were improving asleep. Hang on just a second. It skipped ahead a little bit. Sorry about that.
- Dr. Kristina Simeone: [41:42](#) So, we did another study in which we provoked the seizures. So, we gave a vehicle treatment, and we did a methacholine provocation. We determined how many of the subjects experienced a methacholine-induced seizure. And then a handful of days later, we did the same test except the animals were pretreated with a DORA. So, here is SD20. So, the top row is all the animals that were SD20. SD35 is the middle row, and SD55 is the bottom row here. You can see by SD55, methacholine induces seizures in all of the vehicle-treated epileptic animals.
- Dr. Kristina Simeone: [42:22](#) So, you're able to provoke or trigger a seizure in all of the animals that are at higher risk for SUDEP. Whereas the younger ones, not all of them, methacholine doesn't trigger a seizure in all of them. They're still a little bit protected, which means that there is something here that we can maybe exploit to that might afford seizure protection.
- Dr. Kristina Simeone: [42:44](#) Now, one of the mechanisms that might be causing these seizures is indeed orexin. If we look here, these animals were given a DORA, they were blocking the orexin receptors. And then 30 minutes later, they were exposed to methacholine challenge. So, there's been no sleep that's

occurred. It could significantly reduce the percent of subjects that experienced a methacholine-induced seizure. So, blocking orexin receptors was able to protect against seizures in a subpopulation of these epileptic animals.

- Dr. Kristina Simeone: [43:20](#) So, the last data point I wanted to share with you is that blocking orexin receptors also reduced hypoxia. So, here's just wild type. They don't have very much hypoxia. Our epileptic guys have more hypoxia. When you give a DORA, the hypoxia goes down a little bit in some of the animals. Because blocking the orexin receptor, the hypoxia, we weren't really expecting. We weren't really expecting an effect on the intermittent hypoxia. We got a protective effect. I'll talk about that in just a few minutes as to why I think that might be. But we found that blocking works and receptors did protect a lot of animals against many of these endpoints.
- Dr. Kristina Simeone: [44:09](#) So, next, we determined whether or not we give it daily, if that could increase longevity in this population. So, here's the survival curve. We have the original colony in this gray hatched line. So, this is the survival curve of our original colony. Now, we started the injections in high risk animals. So, we started the injections at P45 for the DORA. You can see that here is the black line.
- Dr. Kristina Simeone: [44:36](#) To properly control for starting the injections at P45, we then created a second survival curve of knockout animals that did not receive any treatment but had lived up until P45. So, this is their survival curve right here in red. So, treating the animals daily with a DORA did increase longevity with this late onset treatment. Whether or not treating the animals sooner will increase longevity even more, postpone sudden death more, and maybe prevent it, that study is the study that needs to be done next.
- Dr. Kristina Simeone: [45:13](#) So, to summarize some of the things I've shared with you today, we found in our preclinical animal model that we do have an intermittent bradycardia. That we have intermittent hypoxia, rapid breathing, apnea, sleep deficiency. They're really, really interdependent, right? So, you can have severe seizure that causes rapid breathing, that causes apnea. The apnea is going to cause intermittent hypoxia, and hypoxia can cause bradycardia.
- Dr. Kristina Simeone: [45:46](#) So, a lot of these nuances, they can influence each other. So, really understanding how much of this is happening in all of the different subjects or all of the different patients, how they're interrelated. Which one happens first? Do any of them happen first? Are these independent ways or methods or biomarkers that may increase SUDEP probability, or do you need to have multiple ones to determine that? All of that still needs to be figured out.

- Dr. Kristina Simeone: [46:14](#) When we blocked orexin receptors, we were able to stabilize heart rate and the animals that have bradycardia. I think maybe reducing that intermittent hypoxia was because they weren't having as many apnea events and they weren't having as many seizures. So, that might be how we were reducing the intermittent hypoxia. It did improve sleep. DORAs are known to improve sleep.
- Dr. Kristina Simeone: [46:36](#) So, this is what we were expecting. We were not expecting it to attenuate seizure severity. So, this is another area of research that we're actively looking at as to how the orexin antagonist was able to attenuate seizure severity. We know that late onset treatment can increase longevity. So, there's just so much more that we need to do. I don't think this is a summary at all. This is more like the beginning of the next chapter or the next book of all of the studies that really do need to be done.
- Dr. Kristina Simeone: [47:07](#) Future impact, I'm hoping next year, we'll have some really interesting responses from the survey. Hopefully, we can now start to determine nodal points of intervention and determine whether preventing any of these biomarkers may increase longevity.
- Dr. Kristina Simeone: [47:23](#) So, I'd like to really thank Stephanie Matthews and Sruthi Iyer. They conducted most of the studies and most of the data that I presented herein. The CURE was foundational in supporting a lot of studies presented herein and also allowed me to get some NIH funding. I also wanted to thank the EFA and other foundations for their funding and support as well. Of course, Tim, who is definitely the harshest scientific critic out of all of my colleagues. So, I wanted to thank everybody. Thank you again for inviting me to speak today, a couple days before SUDEP Action Day. I'll be happy to take any questions now.
- Priya Balasubramanian: [48:09](#) Thanks so much, Kristina. That was a really interesting talk. I think we have some time for questions. But before we begin, I would just like to remind everyone, if you have questions, please do submit them through the Q&A tab. I'll try to get through as many as we can in the remaining time. So, I will start with and I think you might have answered this when you were discussing the REM, non-REM sleep, but the question that came in early on was, "Did you notice in your knockout mice, were the animals having more frequent seizures during the sleep period as they neared SUDEP, or was that not related at all, the sleeping seizures?"
- Dr. Kristina Simeone: [48:51](#) So, that is a fantastic question. That is a study that we haven't done yet. So, the study that looked at the hypnograms, that looked at sleep architecture, we analyzed the data. And then after the study was done, we sacrificed the animals for histological studies. So, we didn't let them live until they died naturally. That study was actually conducted before we started doing that with our endpoints.

- Dr. Kristina Simeone: [49:22](#) So, we know that the animals that had very disrupted sleep that they were a higher risk. They're I think probably SD70, at the age of SD70, where 70% of the knockout colony had passed away or died of sudden death. So, I think that would be a great study. Are there more seizures that are coming out of sleep? Is the sleep architecture disrupted more as within subject as they approach sudden death? We haven't done that. That is a fantastic question.
- Priya Balasubramanian: [49:55](#) Another sleep-related question was, "How do you actually calculate disruption in sleep when how much sleep does a wild type mouse get and then they're going through these bouts of sleeping, waking, the much minimum amount of disruption that's required to increase to SUDEP risk?"
- Dr. Kristina Simeone: [50:13](#) So that's a really great question. Because, yeah, these animals, they're rodents, so they don't sleep for eight hours at a time, right? So, how do you measure sleep deficiency? So, that was a hard question. We analyzed it several different ways. What we ended up with is using actigraphy. So, actigraphy is a non-invasive way of looking at rest and active states. So, these animals were video monitored. They were put into an actigraphy cage, which is an infrared beam that measures activity and rest. They were in that for their entire life. So, we took all of the wild type data.
- Dr. Kristina Simeone: [51:00](#) We found that at the ages we were looking at, there were no changes. It was really, really robust with how many rest EPICs they had during their rest period, during the 12-hour rest period in their light-dark cycle. So, it was constant throughout every single age. So, we took that wild type value of what is standard rest. We compared it to the epileptic animals. We found that as they got closer to death that they had, they had more...
- Dr. Kristina Simeone: [51:35](#) Sorry, I know my arm was not a great line graph. If this was wild type rest, every single day, there was very little variation. If this was the younger knockouts, they look just like the wild type and they had the same amount of rest. And then as they got closer to SUDEP, the rest started to go like this. So, the rest efficiency was this difference between the average all of the wild types together and then each knockout.
- Priya Balasubramanian: [52:02](#) Thank you. And then there's one question about the survival that you talked about, which is very interesting. They want to know, "If you started treating chronically with DORA starting at, say, P30, and then did you happen to see it enhance survival in very large manner, say up to P20 or P50?"
- Dr. Kristina Simeone: [52:29](#) So that's the study that we still need to do is give a DORA... There's lots of different doors out there but give a DORA early on at a much younger age and then see if it can prevent everything from happening and if it can postpone SUDEP. Does that answer the question?

- Priya Balasubramanian: [52:50](#) Yes, I think that answers the question.
- Dr. Kristina Simeone: [52:55](#) So, one thing I'd like to actually expand on that just a little bit is that for research purposes, if you give it before the onset of epilepsy, that's not clinically relevant, right? So, you want to wait until some of the problems have started before you give a drug, so that it's more clinically relevant. But I do think both of them need to be done, both studies need to be done, where you wait for the onset of some of the pathophysiologies to occur and then you give it as a treatment, but then you can actually start a treatment maybe earlier more as a proactive measure.
- Dr. Kristina Simeone: [53:29](#) Now, it's important to know that the DORAs improved sleep. So, you don't want to give it during the period of the day that you're supposed to be awake, because it'll just knock you out and put you to sleep. So, this is really restricted to things that we can only do during the sleep. You can give it at lower doses to help protect against some of these pathologies, and it won't induce sleep, but those are again nuances that we still need to figure out.
- Priya Balasubramanian: [53:59](#) Thanks. I think we have time for maybe one more question. This is a general question. Are there any side effects to doing this chronic treatment with DORA?
- Dr. Kristina Simeone: [54:13](#) So, DORAs are really safe. So, in 2009, when we first started working on these studies, we were using Almorexant, which was just about to be approved by the FDA. It has since been pulled from the market. Suvorexant and Lemborexant are now FDA use drugs. So, now we're using drugs similar to those. So, the benzodiazepine and benzodiazepine-like drugs, when you give them as hypnotics, as a sleep aid drug, they do improve sleep, but they also can cause daytime drowsiness the next day. It can cause other kinds of side effects as well, like cognitive problems the next day and that kind of thing. So, the DORAs are really safe. They're very well tolerated. You don't end up with that next day drowsiness during the day.
- Dr. Kristina Simeone: [55:01](#) So, in terms of their safety profile, clinically, these are very safe drugs so far. They're still relatively new. So, let's wait 5 to 10 years and see what happens. There is something interesting that we just recently found out. We have a study that's actually under review right now, I didn't have time to share this data. But we looked at sleep architecture and EEG of the epileptic animals that had been treated with many different anti-seizure drugs, traditional anti-seizure drugs.
- Dr. Kristina Simeone: [55:30](#) So, in that study, we were able to separate out effects of a drug on sleep versus effects of a drug on a seizure. Because I know a lot of basic scientists and clinicians want to know, "Do the seizures arise more out of REM or non-REM?" We're finding that for at least in our animal model, that the seizures arise both out of non-REM and out of REM just at

baseline, and then the drugs change that up a little bit. Hopefully, that paper will be coming out soon.

Priya Balasubramanian: [56:00](#)

I think that's about all the time we have for questions. I'd like to conclude this virtual seminar. A special thank you to you, Kristina, for this really excellent talk. Also, thank you to the Nussenbaum-Vogelstein Family for their support of the Frontiers in Epilepsy Seminar series. I'd like to thank everyone in our audience today for asking all of these great questions and for your participation.

Priya Balasubramanian: [56:27](#)

If you'd like more information about hosting a seminar series at your institution or about applying to one of our CURE Epilepsy grants, please visit our For Researchers page on our website, cureepilepsy.org, or you can write to us at research@cureepilepsy.org. We have one more seminar coming up, which is entitled TANGO: A novel therapeutic approach to treat SCN1A-linked Dravet Syndrome, which will be presented by Dr. Isom from the University of Michigan Medical school. Finally, I'd like to request you all to please fill out the short survey that will appear at the end of this webinar. Once again, thank you all and stay safe.